PRODUCTION AND NUTRITION RECOVERY OF CROPS IN A RECIRCULATING AQUAPONIC SYSTEMS
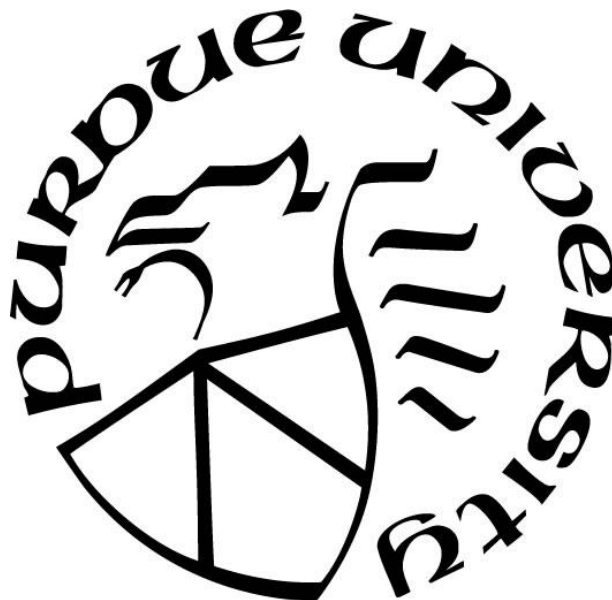
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Dedicated to my beloved family and all my friends

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ABSTRACT

The goal of this research was to improve crop yield and quality and enhance nutrient use efficiency of aquaponics for the development of sustainable aquaponic production system. Aquaponics is the integration of aquaculture and hydroponics by recirculating water and residual nutrients resulting from aquaculture wastewater into hydroponic crop production. The project had four objectives. The first objective was to characterize nutrient composition and accumulation in recirculating water and plant parts of tomato, basil, and lettuce grown in aquaponic systems, and to compare their growth and yield with those grown in hydroponic systems. The second objective was to determine the effects of feeding management regime on water quality, crop yield and quality, and N use efficiency for vegetable and herb production in recirculating aquaponics in comparison to hydroponics. The third objective was to optimize water-flow rate for efficient aquaponic system for maximum crop yield. The fourth objective was to investigate and compare the N and P mass balance between aquaponics and hydroponics. Four conclusions were determined that 1) Aquaponic solution was deficient in Ca and/or Mg leading to plant nutrient deficiency but sufficient or high in P; And luxuriant nutrient profiles in hydroponics are not necessary to enhance crop yield in aquaponics as long as key factors affecting crop yield are identified and properly addressed. 2) Uniform feeding regime improved water quality by reducing toxic ions and enhancing initial nutrient availability and considerably increased the yield, quality and nitrogen use efficiency (NUE) of crops in aquaponics as close or similar to those in hydroponics. 3) Flow rate is an important factor affecting water quality parameters and optimizing flow rate is essential to maximize aquaponic crop production and improve energy efficiency; High hydraulic loading rate at 3.3 m^3/m^2-day improved performance and yield of all crops in an aquaponics system regardless of their growth rate, but the water hydraulic loading rate for fast-growing and medium-growing crops can be reduced to 2.2 m^3/m^2-day without production reduction. 4) Plant species had significant influence on N and P removal and mass balance in aquaponics and hydroponics; Fruity vegetables showed better growth adaption in aquaponic system, while yields of leafy vegetables may be reduced when grown in aquaponics than hydroponics; Aquaponics is more efficient than hydroponics releasing less environmental wastes, however, N and P use efficiency in aquaponics and hydroponics can be further improved via proper management.

The important findings obtained from this research will fill the knowledge gap in aquaponic research and provide new management strategies to improve quantitative study of aquaponic crop production and new management strategies for cultivating crops in aquaponics. The findings will also greatly contribute to the commercial aquaponic development, and ultimately improve food security and resource use efficiency in the US and global agricultural production.

GENERAL INTRODUCTION

1. Background information

The UN's World Population Prospects 2019 estimated that the world's population will rise from 7.7 billion to approximately 9.7 billion by 2500 and stated that the rapid population growth presents challenges for sustainable development (United Nations, Department of Economic and Social Affairs, Population Division, 2019). "Sustainable development is development that meets the needs of the present, without compromising the ability of future generations to meet their own needs (World Commission on Environment and Development, 1987)." Facing the rapid increase of population with diminishing water, land and energy resources, sustainable development could be achieved by supporting the expansion of sustainable food production on land and in water, which requires enhancing the efficiency of agricultural and aquacultural production with less energy cost (Cash et al., 2003).

Currently, hydroponic systems are known as one of the most efficient systems for agricultural production (Takahashi et al., 2018; Treftz & Omaye, 2016). Hydroponic systems culture plant crops in soilless nutrient solution rather than soils. Compared to traditional farming, hydroponic production has various advantages including less water and nutrient consumption, better environment control, less soil-borne disease and pest infection, and higher yields and better quality products (Lommen, 2007; Molitor, 1990). In recent years, hydroponic crop production has dramatically increased in the U.S. (Van Patten, 2008). It was reported that the number of the present commercial hydroponics enterprises is 5 times higher than that of 10 years ago and its global value is nearly $8 billion US dollars (Carruthers, 2002; Lee & Lee, 2015). However, after decades of research and application, there are still some limitations in hydroponic production that have not been solved, which include high setup cost, rapid spread of water-borne diseases, and a demand for specialized management knowledge (Lee & Lee, 2015). The most important issue is wastewater management and disposal. It was reported that nearly 90% of hydroponics operations are open system, which means that nutrient-rich wastewater is disposed to the environment (Jensen, 1997). Even in a closed hydroponic environment, nutrient solution needs to be exchanged regularly as a result of nutrient imbalances (Thiyagarajan, Umadevi, & Ramesh, 2007). Thus, more efficient wastewater management have become imperatives for the future agricultural production using hydroponic systems.

Meanwhile, aquacultural production is being challenged by rising consumer demand and continues wild fish stocks declining as a result of overfishing (Adler, Harper, Wade, Takeda, & Summerfelt, 2000). In order to meet the demand of fish or fish by-products and relieve the stress for wild fisheries, further expansion of sustainable aquaculture industry is necessary. The Food and Agriculture Organization of the United Nations (Moffitt & Cajas-Cano, 2014) reported that aquaculture has become one of the fastest-growing segments in food production and contributed approximately 50% of the whole fish and fish products for nearly 7.3 billion people with an average of one-fifth of total animal protein intake. This will only continue to rise as demand for seafood is increasing and fisheries are being depleted. Consequently, this intensive aquaculture

industry will inevitably have significant environmental impacts. It was reported that total fresh water withdrawal for aquaculture in 2010 was about 3.6×10^{10} L/day (Goddek et al., 2015; Maupin et al., 2014). For aquaculture production generates, 10 to 20% daily disposal of wastewater is necessary to maintain water quality and fish health. The nutrient-rich wastewater is high in nitrogen (N) and phosphorus (P). These are the main end-products of aquaculture, which not only deteriorate water quality in the rearing tank, but also the environment as a whole (Lazzari & Baldisserotto, 2008), such as eutrophication and a consequent change in the aquatic ecosystem (Jahan, Watanabe, Kiron, & Satoh, 2003).

Facing challenges in current agricultural and aquacultural production, aquaponics has emerged as an important food production system. Aquaponics integrates aquaculture and hydroponics by recirculating water and residual nutrients resulting from aquaculture wastewater into hydroponic crop production. The basic concept of an aquaponic system is a sustainable, controlled production system that imitates natural biological cycle, minimizes the usage of nonrenewable resources, and provides the potential to increase economic profits (Tyson, Treadwell, & Simonne, 2011). Unlike hydroponics or aquaculture systems alone, fresh water and nutrients added into an aquaponic system are recycled for fish and plant production, thus aquaponics also is called a "zero-discharge system" (Boxman, 2015). In addition to the merits inherited from the individual systems, aquaponics has a great advantage in managing wastewater, and therefore solving the biggest issue in current food production. However, there are challenges in aquaponic crop production, such as crop growth reduction of both fish and plant crops due to the unfavorable growth environment for the production of each crop, the accumulation of nitrogen compounds as a result of insufficient nitrification (Wongkiew, Hu, Chandran, Lee, & Khanal, 2017) and many unanswered questions regarding management requirements (optimum water quality and chemistry, biofilter design, feeding rate, pumping water flow rate, etc.) (Tyson et al., 2011). The efficiency of aquaponics appears to be higher than that of hydroponics; however, limited scientific investigations have been made so far to verify in this aspect. There is lack of quantitative research to support the development of sustainable and economically feasible aquaponic systems (Goddek et al., 2015). Various factors (e.g., nutrient (fish feed) input, water quality, crop species, microbes, culture environment, management strategies) can affect the aquaponics efficiency and crop yield, and therefore, it is critical to unravel the complex mechanism of aquaponics to improve crop production and quality.

2. Current research status

The concept of aquaponics was first conceived by aquaculture producers (Graber & Junge, 2009; Rakocy, 1993; Timmons, Ebeling, Wheaton, Summerfelt, & Vinci, 2002). This is a recycling system combining aquaculture and hydroponics–cultivating crops in hydroponics sub-system reuses the wastewater from aquaculture sub-system. The toxic constituents (ammonium and nitrite) in wastewater are converted to ammonium and nitrite to nitrate by nitrifying bacteria via nitrification process, and then nitrate and other nutrients dissolved in aquaculture wastewater are taken up by plant crops. The clean water then flows back to aquaculture sub-system or fish tank. With this recycle mechanism, aquaponics can double the use of aquaculture wastewater and make

it into at least two cash crop production, fish and plant crops (Diver, 2006; Rana et al., 2011; Tyson et al., 2011).

Compared to individual systems, aquaponics has great advantages. First, it is environmentally sustainable because no wastewater is discharged to the environment. Contrarily, both aquaculture and hydroponics generate a large volume of wastewater or spent nutrient solution and it should be replaced with freshwater in order to resume the production. The wastewater from aquaculture and hydroponics contains substantial amounts of uneaten feed or nutrient residuals, particularly N and P, environmental pollutants, and these nutrients are associated with eutrophication and other environmental issues. Recently, many countries have developed the laws and regulations to control wastewater disposal issues in aquaculture and hydroponics production (Chávez-Crooker & Obreque-Contreras, 2010; Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2007; Gray, Wu, & Or, 2002; Holmer & Kristensen, 1992). Aquaponics can solve this issue by producing crops with zero or minimal release of nutrient-rich wastewater. Second, aquaponics can harvest fish as an additional food or revenue source. Unlike traditional agriculture production, aquaponics can culture two cash crops simultaneously as it couples soilless crop production with aquaculture production. Presently, the feasibility of integrated production of fish and plant has been demonstrated (Buzby, Waterland, Semmens, & Lin, 2016; Pantanella, 2012; Rakocy, Bailey, Shultz, & Thoman, 2004; Rana et al., 2011) and some combinations of vegetables (lettuce, basil, tomato, et al.) and aquatic animals (tilapia, trout or ornamental fish, et al.) have been tested in aquaponics. Therefore, it can increase the potential for higher income. Third, aquaponics can be set up in any kind of environment if a minimal amount of water and adequate energy are provided. Thus, aquaponics can address high food demands associated with increasing global population and decreasing natural recourses.

Although it is a promising food production system, there are many unknowns that make it difficult to make aquaponic crop production profitable, which need to be properly addressed. Researchers found that not all crops can be cultured successfully in aquaponics (Buzby et al., 2016). Basic knowledge and scientific information are critical for the establishment of aquaponics and successful operation (Love et al., 2014). Some management factors have been investigated to improve aquaponic crop production, e.g. foliar application (Roosta & Hamidpour, 2011), length of gully (Khater & Ali, 2015), flow rate (Nuwansi et al., 2016), and crop performance in comparison with hydroponics (Pantanella, 2012; Saha, Monroe, & Day, 2016; Suhl et al., 2016; Wortman, 2015). However, these studies lacked quantitative analytical data to answer the causes of crop reduction in aquaponics. Therefore, the results cannot be applicable to commercial aquaponic production. In addition, the information on the interaction between water quality parameters and crop performance in aquaponics is limited, which is critical to improve aquaponic system efficiency. Our study aimed at investigating the dynamic changes in water quality in association with crop growth in aquaponics in comparison to hydroponics and developing practical management strategies to increase crop yield and quality.

3. Review of relevant literature

3.1. Aquaponics nutrient availability and crop production

There are three organisms in aquaponics: plants, fish and nitrifying bacteria. Current water quality parameters recommended for aquaponic crop production is designed to maintain fish health rather than produce plant crops. This is a challenge for the aquaponic industry as a major income source in aquaponics is plant crops (Quagrainie, Flores, Kim, & McClain, 2018). General water quality parameters for each organism are listed in **Table 1**.

Table 1. General water quality tolerances for crops, fish and bacterial colony (Carruthers, 2015).

Organism Groups	pH Requirement	Temperature Range (°C)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	DO (mg/L)
Warm water fish	6-8.5	22-32	<3	<1	<400	4-6
Cold water fish	6-8.5	10-18	<1	<0.1	<400	6-8
Plants	5.5-7.5	16-30	<30	<1	-	>3
Bacteria	6-8.5	14-34	<3	<1	-	4-8

In aquaponics, most of the nutrients provided for plant growth come from fish feed. Most fish species only absorb 20–30% of nitrogen in the diet, but release the rest 70–80% as waste into water, which could be a potential nutrient source for crop growth (Krom, Ellner, van Rijn, & Neori, 1995; Rakocy, 1993; Roosta & Hamidpour, 2011; Schneider, Sereti, Eding, & Verreth, 2005). However, some critical nutrient elements (potassium, iron, manganese, etc.) required for plant crops are missing in fish feed. Thus, fertilizer supplementation is suggested in aquaponic systems (Delaide, Goddek, Gott, Soyeurt, & Jijakli, 2016; Graber & Junge, 2009). Roosta & Hamidpour (2011, 2013) found that foliar spray of potassium (K), iron (Fe), manganese (Mg), zinc (Zn), magnesium (Mn) and boron (B) increased tomato production in the order of: K> Fe >Mn >Zn >Mg > B. Roosta & Mohsenian (2012) also found that foliar spray of different iron sources ($FeSO_4$, Fe-EDTA and Fe-EDDHA) helped overcome iron deficiency in aquaponics. In addition, Roosta (2014) suggested foliar spray of potassium in aquaponics as it effectively alleviated magnesium, manganese and zinc deficiency, and sodium toxicity.

Except foliar spray, very limited research had been conducted using other types of application methods to supplement nutrients. Ru et al. (2017) found that the addition of macro- and micro-nutrients increased the growth of crop and fish when the nutrient solution was directly added into aquaponic systems. Zou et al. (2017) found that adding exogenous carbon (polylactic acid) could decrease the denitrification and increase plant production by increasing the fermentation products of lactic acid. Cerozi & Fitzsimmons (2017) used dietary phytase supplemented fish feed in aquaponics and found that phytase supplementation increased accumulation of phosphorus in fish carcass without affecting plant growth performance.

In summary, external nutrient addition was suggested as one of the practical strategies to increase plant crop production in aquaponic systems.

3.2. Fish species in aquaponics

According to an international survey, more than a dozen of fish species have been raised in aquaponic systems (Love et al., 2014). Tilapia (*Oreochromis niloticus*) is a warm-water species that is the most popularly used in aquaponic studies and the most commonly grown in commercial aquaponic systems in the U.S. This is because of its excellent adaptability to a fluctuating water environment including temperature, pH, dissolved oxygen (DO), and total ammonium nitrogen (TAN). Temperature requirement of tilapia does not match with that of many plant crops popularly grown in aquaponic systems. Most of the economically important vegetables are cool-season crops and may perform less than optimally in warm-water based aquaponic systems; however, there are nearly no reports investigating the thermal effects (Bakhsh, Chopin, Murray, Belyea, & Hamer, 2015). Trout and shrimp have received interests from many researchers in recent years (Pinheiro et al., 2017; Sace & Fitzsimmons, 2013). Ornamental fish and catfish are other popular fish species in commercial aquaponics (Love et al., 2014). Other fish species used include perch, bluegill, bass, and crawfish (Love et al., 2014). However, limited scientific research have been conducted to compare the different combinations of fish and plant species, and therefore their interactions and synergistic effects are still unknown.

3.3. Crop performance in aquaponics

As the "biological filters" in aquaponics, different crop species play an important role in the aquaponic system performance. Liang & Chien (2015) indicated that the photosynthetic efficiency of plants greatly affects water quality and fish production of aquaponics. Leafy vegetables are the most popular crop species grown in aquaponics. Lettuce (*Lactuca sativa*) is a cool-season crop and the most widely cultivated vegetable in aquaponics (Blidariu, Dra, & Grozea, 2013; Buzby et al., 2016; Mangmang, Deaker, & Rogers, 2015). Other leafy vegetables widely employed in aquaponic research include mustard greens (*Brassica juncea*), water spinach (*Ipomoea aquatica*), coriander (*Coriandrum sativum*), pak choi (*Brassica rapa* subsp. chinensis), and Swiss chard (*Beta vulgaris*) (Buzby et al., 2016; Hu et al., 2015). Johnson (2014) found that only 'Speckled Amish' among 28 lettuce cultivars showed higher productivity in aquaponics compared to hydroponic treatment. Hu et al. (2015) found that tomato-based aquaponics showed 4.2-folds higher amount of nitrifying bacteria on the root surface and better water quality (lower ammonium and nitrite concentrations) than pak choi-based aquaponics. Buzby et al. (2016) cultured 34 leafy vegetable and herbs in a low nutrient aquaponic system and found that there was no significant difference in stand establishment among crop species. Some research used halophytes (*Sesuvium portulacastrum*, *Batis maritima* and *Salicornia europaea*) in aquaponic systems and found that marine (or salt water) aquaponics could be a strategy to manage nutrient removal (Boxman et al., 2017; Webb et al., 2012).

Most aquaponics studies focus on crop selection within leafy vegetables (Pantanella, 2012; Roosta, 2014) and there are still limited information on fruity (Graber & Junge, 2009; Roosta & Hamidpour, 2013) and herb (Mangmang, Deaker, & Rogers, 2016) crops.

Herb crops tested in aquaponic systems include basil (*Ocimum basilicum* L.), peppermint (*Mentha × piperita*), and spearmint (*Mentha spicata*) (Espinosa Moya et al., 2016; Roosta, 2014; Saha et

al., 2016). Espinosa Moya et al. (2016) cultured basil, peppermint, and spearmint in tilapia-based aquaponics and found that the herbaceous plants removed ammonium and phosphate by 45-50% and 55-60%, respectively, but there was no significant difference among species.

Fruity vegetables (Hu et al., 2015; Knaus & Palm, 2017a, 2017b; Mariscal-Lagarda et al., 2012; Villarroel, Alvariño, & Duran, 2011; Villarroel et al., 2016) used in aquaponics research include strawberry (*Fragaria × ananassa*), tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus* L.). Researchers (Graber & Junge, 2009; Hu et al., 2015) found that tomato and cucumber showed good performance (nitrogen utilization efficiency, abundance of nitrifying bacteria, and water quality) in aquaponics. Knaus & Palm (2017b) found that different fish species effected crop growth performance and cucumber showed better performance in combination with Common carp (*Cyprinus carpio*), while tomato showed better performance in combination with Nile tilapia (*Oreochromis niloticus*). Villarroel et al. (2011) suggest that it is feasible to integrate low-density fish culture for strawberry production in aquaponics.

Since most of the mentioned studies have not directly compared plant growth and performance among different types of vegetable crops, it is not clear which vegetable crops perform better in the aquaponic system.

3.4. Production strategies

In aquaponics, all nutrient inputs come from fish feed (and nutrient fertilizer in some aquaponic systems supplemented with fertilizer), thus production strategy is a critical part of aquaponic system management. In the study examining seedling establishment of 34 cool-season vegetables in flow-through fish culture systems with low nutrient-profile, Buzby et al. (2016) found that there was no significant difference in seedling-stand establishment (percent-filled cells) among plant species. They also found no significant effects of flow rates: low (18.9 L/min) and high (75.7 L/min) on crop production. In their other study comparing the yield of lettuce grown with difference sources of aquaponic solutions, nitrate and phosphate concentrations in the system ranged from 0.2 to 0.6 mg/L and from 0.1 to 0.25 mg/L, respectively (Buzby & Lin, 2014), which were 10 to 100-fold lower than those found in a typical recirculating fish-culture system. Lack of the growth differences among the vegetable species in their studies might be due to the extremely low nutrient concentrations in their system. Contrarily, some studies using high nutrient profile an aquaponic system showed that flow rate (or hydraulic loading rate) and nutrient availability affected crop yields. Endut, Jusoh, Ali, Wan Nik, & Hassan (2010) found that fish production, water spinach growth, and nutrient removal efficiency were the highest at hydraulic loading rate of 1.28 m/day. Diessner (2013) found that stocking density had no significant effect on crop yields of lettuce and pak choi, but nutrient content in the leaves of vegetables was increased with higher stocking density. Mariscal-Lagarda et al. (2012) found there was no significant difference between tomato yields irrigated with white shrimp (*Litopenaeus vannamei*) effluent and those with nutrient solution. There is limited information on quantitative comparison of combined production strategies.

3.5. Dissertation organization

The following chapters of this dissertation reported the findings of four separate studies to address the main objectives highlighted in the general introduction: i) Characterizing nutrient composition and accumulation in tomato-, basil-, and lettuce-based aquaponic and hydroponic systems; ii) Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems; iii) Effects of flow rate on spatial and temporal changes in water quality and crop growth and yield in aquaponic systems; and iv) Comparisons of N and P mass balance of lettuce-, basil-, and tomato-based aquaponics and hydroponics. Each manuscript included a separate Introduction, Material and Methods, Results, Discussion and Conclusion sections. The dissertation author was responsible for conducting all experiments described in the four manuscripts that comprise the chapters of this dissertation.

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CHARACTERIZING NUTRIENT COMPOSITION AND ACCUMULATION IN TOMATO-, BASIL-, AND LETTUCE-BASED AQUAPONIC AND HYDROPONIC SYSTEMS

Abstract

Aquaponics studies often utilize water and/or nutrient sources that are not well-defined, providing incomplete information about nutrient profiles in the system. This study was conducted to characterize nutrient composition and accumulation in recirculating water and plant tissues in tilapia-based aquaponics filled with reverse osmosis water and to determine their subsequent effects on growth and yield of cherry tomato, basil, and lettuce as compared to those in hydroponics. Daily nutrient release rates from ingested fish feed were in decreasing order of macronutrients: SO_4–S > NO_3–N > PO_4–P > K > Cl > Ca > Na > NH_4–N > NO_2–N > Mg and their average concentrations during 3-month production were significantly lower in aquaponics than in hydroponics, with exception of NO_2-N, NH_4-N, Na and Cl. Ca and Mg concentrations were substantially low in aquaponic solution especially toward the end of production. P concentrations averaged 30 mg L^{-1} and gradually increased over time regardless of vegetable type. Nutrient accumulation in aquaponic solution was the lowest in the tomato-based system, followed by basil- and lettuce-based systems. All plants grown in aquaponics contained less total N in the leaves compared to those in hydroponics, in addition to less Ca and Mg in tomato and Mg in basil. In contrast, lettuce grown in hydroponics developed Ca deficiency due to relatively faster biomass production but not in aquaponics. Regardless of nutrient status, tomato grown in aquaponics produced the same fruit yield as that in hydroponics but with significantly reduced vegetative shoot biomass, while basil and lettuce grown in aquaponics produced significantly lower marketable yields compared to hydroponics. Our results highlight nutrient composition and accumulation in aquaponic systems and provide potential solutions to design proper nutrient management practices essential for the improvement of crop production and the development of efficient aquaponic systems.

1. Introduction

Aquaponics is an integrated system that combines aquaculture and hydroponics, in which water from the fish tanks enriched with mineral nutrients is used to produce plants. Disposal of considerable amounts of nutrient-rich wastewater is a significant problem in fish cultivation, as it is associated with surface and groundwater pollution (Abdel-Raouf et al. 2012). Aquaponics not only eliminates wastewater discharge issues, but also enables spent water and nutrients to recirculate in the system because hydroponic component in an aquaponic system performs as biofilter and effectively controls the accumulation of waste nutrients from fish culture (Rakocy and Hargreaves 1993). As such, fish and vegetable production in a recirculating aquaponic system can achieve a high degree of efficiency of water use (McMurtry et al. 1997), while allowing production of additional saleable crops (Rakocy and Hargreaves 1993). Aquaponics has great potential to contribute to both global and urban sustainable food production and to reduce

environmental impacts associated with agricultural production. However, commercial aquaponics is a nascent industry and its nutrient management, crop production, and technical aspects have yet to be optimized.

Chemical fertilizers commonly used in hydroponics either require intensive energy inputs for synthesis (i.e., N fertilizer), are derived from nonrenewable resources (i.e., phosphorus), and/or generate a large carbon footprint for transport (Goddek et al. 2015). Recirculating aquaponic systems are known to save not only the use of chemical fertilizers for crop production but also 90 to 98% water compared to field production (Al-Hafedh et al. 2008). Hydroponic nutrient solutions are formulated to contain luxuriant nutrient concentrations in inorganic forms, making them readily and abundantly available for plant growth (Resh 2013), and this is considered to be important for intensive hydroponic systems with high production yield. Hydroponic solutions are regularly adjusted to a desirable electrical conductivity (EC) suggested for the production of a particular crop. While this does not guarantee the presence of individual nutrient elements, it is a simple, commonly used method to ensure total soluble salts in the nutrient solution.

In aquaponics, nutrient composition of fish feed has major influence on nutrient composition and concentration in aquaponic solution, directly affecting water chemistry (Rakocy and Hargreaves 1993) and plant growth (Pantanella et al. 2012). Fish waste released to aquatic phase is known to contain sufficient levels of mineral nutrients necessary for plant growth, except calcium (Ca), potassium (K), and iron (Fe) which are deficient in aquaponic solution due to the suboptimal level of such in fish feed (Rakocy et al. 2004). Therefore, it is suggested to be supplemented to aquaponic solution as potassium hydroxide and iron chelates or applied as a folia spray (Rakocy et al. 2004; Rakocy et al. 2006; Roosta and Hamidpour 2011). Phosphorus (P) is also reported to be deficient in aquaponics due to the precipitation with Ca (Goddek et al. 2015; Savidov et al. 2007; Seawright et al. 1998). Meanwhile, aquaponic solution is reported to contain high Na levels, which was considered primarily due to the use of Na-containing solution for pH correction (Seawright et al. 1998; Delaide et al. 2016) and fish feed. In fact, most of aquaponic nutrient studies have utilized various water sources that are not well defined, which makes it difficult to generalize such research findings as nutrient profiles are not only derived from fish feed but also contributed by background nutrients contained in the water source. The incomplete information on available nutrients in aquaponic solutions makes it difficult to properly design nutrient management practices essential for the improvement of plant and fish yield in aquaponic systems.

Accordingly, crop growth and yield in aquaponic systems have been reported to be inconsistent among aquaponic studies ranging from lower to higher compared to those in hydroponics. For example, basil in aquaponics produced lower yield compared to hydroponics (tap water was used) and developed nutrient deficiency symptoms, which was considered due to lower Fe, Mn, and K concentrations (Roosta 2014). Marketable yields of leafy vegetables (i.e., basil and kale) and fruity vegetables (i.e., tomato and pepper) were significantly lower in a simulated aquaponic solution (low EC and high pH; well water was used) compared to those grown in hydroponics (Wortman 2015). In their study, vegetative growth (nonmarketable yield) of tomato and pepper tended to be lower in aquaponics. In contrast, growth and yield of lettuce grown in aquaponics was higher than those grown in hydroponics (tap water was used) (Alcarraz et al. 2018). Yields of tomato and mini-

cucumber in aquaponics (freshwater was used) exceeded average yields of those in commercial greenhouse using conventional hydroponics (Savidov et al. 2007), although direct yield comparisons between aquaponics and hydroponics were not made in this study. Meanwhile, growth and yield of lettuce in aquaponics was similar to that of hydroponics (Delaide et al. 2016; Pantanella et al. 2012) (rainwater and distilled water was used, respectively). Fruit yields of cucumber, tomato, and eggplant were not different between aquaponics and hydroponics; however, vegetative growth was not reported in their 1-, 2- and 3-month production point, respectively (Graber and Junge 2009) (tap water was used). There was no difference in fruit number and yield of tomato grown in aquaponics and hydroponics although significant reduction of vegetative growth was observed in tomato grown in aquaponics without visible signs of nutrient deficiency (tap water was used) (Roosta and Hamidpour 2011). Similarly, yield and growth of tomato grown in double recirculating aquaponics was not different from that in hydroponics, except reduced leaf area (freshwater containing high levels of Ca and Mg was used) (Suhl et al. 2016).

Such discrepancy among the aforementioned studies in aquaponic crop performance and yield may reflect much of the variation in nutrient profile of aquaponic solution, which can be affected by water source used for aquaponic systems. Characterizing nutrient profiles directly derived from fish feed and nutrient accumulations in water and plants will aid in designing proper nutrient management practices to improve plant and fish yield, leading to the development of efficient aquaponic systems. Therefore, the objectives of our study were to characterize nutrient composition and accumulation in recirculating water and plant parts of tomato, basil, and lettuce grown in aquaponic systems, and to compare their growth and yield with those grown in hydroponic systems. We determined daily nutrient release rates from fish feed and dynamic changes in nutrient levels in aquaponics by using water source with relatively minimal nutrient contribution to aquaponic nutrient solution. This research will provide critical information on nutrient availability in aquaponic systems and help enhance plant production and yield through proper nutrient management practices.

2. Materials and methods

2.1. Experimental setup and operation

Six experimental units were operated in the greenhouse in West Lafayette, IN (lat. 40°N, long. 86°W).

Each aquaponic unit was equipped with a fish tank (350 L), a clarifier (20 L), two biofilter tanks (20 L each), and a deep-water hydroponic culture unit (350 L; 1.0 m^2) (**Figure 1 A**). Three weeks prior to the study, the aquaponic systems were filled with reverse osmosis (RO) water and its nutrient profile is presented in **Table 2**. Nile tilapia (*Oreochromis niloticus* L.) fish were obtained from Animal Sciences Research and Education Center at Purdue University which had been cultivated in conventional aquaculture system for 4-months. At the time of receipt, fish fresh weight was measured and evenly distributed to three different fish tanks with a stocking density of 20 kg m^{-3} each (average fish weight of 300 g) to raise the electrical conductivity (EC) and establish microbial community including nitrifying bacteria for one month prior to the study.

The pH of the aquaponic systems was maintained at 6.5 to 7.0 using a combination of KOH, Ca(OH)$_2$, and NaOH. During a 3-month production period, a complete diet (41% protein, 1.1% phosphorus) with 4.8-mm floating pellets was used. Fish was fed daily at 9:00 am with fish feed (AquaMax Sport Fish 500, Purina Mills, St. Louis, MO) at 1% of body fresh weight.

A peristaltic pump (Masterflex, Cole-Parmer, USA) was used to recirculate nutrient solutions within a system unit. The total water volume in an aquaponic and hydroponic unit was 700 L with a flow rate of 138 L h^{-1}, giving a water retention time of 300 min in fish tank/nutrient solution reservoir and in floating system unit. Water in the clarifier captured majority of suspended solids from the fish tank. After passing through the clarifier, the aquaculture effluent or nutrient solution flowed into the biofilter filled with biomedia (K1 filter media) and then the hydroponic unit. Plants were held up by a foam board which was set on the top edges of the hydroponic unit. An air pump was used to provide and maintain dissolved oxygen (DO) concentrations above 5 mg L^{-1} to each of hydroponic culture unit and fish tank (or nutrient solution reservoir) using three air diffusers. The fish tanks and nutrient solution reservoirs were covered with a high-density polyethylene (HDPE) board with an opening to permit light to the tank during daytime. Nutrients dissolved in aquaponics or hydroponics were absorbed by plants in the hydroponic unit and reclaimed water was then recirculated into the fish tank/nutrient-solution reservoir. The water was recirculated continuously within each unit, and no water exchange was performed over the experimental period except for replenishing evapotranspiration losses with reverse-osmosis water. Water temperature was maintained within a target range (26–28°C) using an aquarium heater (Eheim Jager TruTemp, Germany) in the fish tank for tilapia culture in aquaponic systems, while it was not controlled in hydroponic systems.

Similarly, each hydroponic system was equipped with a nutrient reservoir (350 L), a clarifier (20 L), two biofilter tanks (20 L each), and a deep-water hydroponic culture unit (350 L; 1.0 m^2) (**Figure 1 B**). The basic configuration of hydroponics was the same as for aquaponics. In each hydroponic culture unit, the nutrient-solution reservoir and each hydroponic-culture unit were filled with reverse-osmosis water blended with nutrient stock solution at 1:100 dilution rate (**Table 2**) which was used as initial and follow-up daily replenishment for fruity vegetables (CropKing, Lodi, OH, US), and leafy/herb vegetables (CropKing, Lodi, OH, US). The EC was maintained at 2.0 mS cm^{-1} by adding and replenishing nutrient solution daily. The pH of hydroponics was adjusted to approximately 6.0.

The photoperiod was 14-h (8:00 am to 10:00 pm) consisting of natural daylight with supplemental lighting using high-pressure sodium (HPS) lamps (600-W, P.L. Light Systems Inc., Beamsville, ON, Canada). A supplemental photosynthetic photon flux (PPF) of the greenhouse was measured using a quantum sensor (LI-250A light meter; LI-COR Biosciences, Lincoln, NE) and photosynthetically active radiation in the greenhouse was averaged at 168 µmol m^{-2} s^{-1}. Day (8:00 am to 10:00 pm) and night (10:00 pm to 8:00 am) temperatures were set at 24 and 18°C, respectively, with an hour transition between the two temperature regimes. Depending on ambient temperature, the greenhouse was cooled as needed using a pad-and-fan evaporative-cooling system, heated using radiant hot-water-pipe heating, and retractable shade curtains regulated by an environmental control system (Maximizer Precision 10, Priva Computers Inc., Vineland Station,

ON, Canada). Environmental data for greenhouse ambient daily light integral (DLI), ambient temperature, and vapor pressure deficit (VPD) were averaged per day and presented in **Figure S1**.

2.2. Plant and fish materials

In this study, cherry tomato (*Lycopersicon esculentum* 'Washington Cherry'), basil (*Ocimum basilicum* 'Genovese'), and lettuce (*Lactuca sativa* 'Cherokee') were selected for the variation in their production duration and harvest method due to the differences in plant parts consumed. For example, tomato plants were continuously grown to maturity and only fruits are removed, leaving intact roots and shoots in the system, which allowed continuous removal of nutrients from system. Basil plants were cut back at maturity and harvested only the top portion of vegetative shoots on a regular basis, allowing the shoots to grow back and roots continue to grow. Meanwhile, lettuce plants were grown for approximately 30d, mature plants were completely removed and new lettuce seedlings were transplanted. Seeds were purchased from a commercial source (Johnny's Selected Seeds, Winslow, ME) and sown in Agrifoam soilless plugs (SteadyGROWpro, Syndicate Sales, Kokomo, IN) with few days interval to match the size of seedlings at the time of transplanting. Seeds were initially imbibed with tap water, followed by gradually increasing to a half-strength fertilizer solution once germinated, and full-strength fertilizer after seedlings develop true leaves (Kim et al. 2018b). The fertilizer was irrigated as necessary with a combination of two water-soluble fertilizers (3:1 mixture of 15N–2.2P–12.5K Cal-Mag Special and 21N–2.2P–16.6K Multi-Purpose fertilizers, respectively; Everris NA, Dublin, OH). The fertilizer consisted of (mg L^{-1}): 150 nitrogen (N), 20 phosphorous (P), 122 potassium (K), 38 calcium (Ca), 15 magnesium (Mg), 0.8 iron (Fe), 0.4 manganese (Mn) and zinc (Zn), 0.2 copper (Cu) and boron (B), and 0.1 molybdenum (Mo). The nitrate form was 76% of nitrogen provided. After the third true leaf of seedlings emerged, uniform healthy seedlings were chosen and transplanted into mesh pots (diameter: 7.6 cm, height: 6.4 cm) each containing 85 g clay pebbles, then transferred to a hydroponic unit of aquaponic or hydroponic systems. There were 8 plants per unit for cherry tomato and 24 plants per unit for basil and lettuce.

Tomato plants were trellised to overhead wires and pruned to a double-headed stem. All suckers developing between the axis of the leaf and stem were removed weekly. Self-pollinations were manually performed daily by agitating flowers with a battery-operated pollinating tool (VBP-01 Garden Pollinator, VegiBee, Maryland Heights, MO).

2.3. Water parameter measurements

Water-quality parameters were monitored in each aquaponic and hydroponic unit, which include dissolved oxygen (DO), temperature, pH, and electricity conductivity (EC) of fish tank (or nutrient reservoir in hydroponic system) and hydroponic culture unit daily at 9:00 am before feeding using the HQ40d Portable Water Quality Lab Package (HACH Corp., Loveland, CO, USA).

Water samples were collected from fish tank (or nutrient reservoir) and hydroponic culture unit once every 4 days before feeding to monitor total ammonia nitrogen (TAN), nitrite (NO_2–N), nitrate (NO_3–N) and phosphate (PO_4–P) concentrations, and were analyzed immediately using HACH reaction kits (Loveland, Co. Ltd., USA), namely Ammonia Reagent Powder Pillows,

Nitrite Reagent Powder Pillows, Nitrate Reagent Powder Pillows, and Phosphate Reagent Powder Pillows, respectively. This activity was performed to ensure fish health and wellbeing and maintain water quality. The same water samples were used to analyze macronutrients (i.e., NO_3–N, NO_2–N, NH_4–N, PO_4–P, K Ca, Mg, SO_4–S, Na, Cl) by ion chromatography (Dionex ICS–5000, Thermo scientific, Co. Ltd., USA) as described below (2.6).

2.4. Daily nutrient release measurements

Daily nutrient release rate of fish was examined by placing tilapia fish (stocking density: 20 kg m^{-3}) in a 350-L tank without plants using recirculating aquaponic systems (**Figure 1 A**). The water condition was maintained the same as the operating conditions as described above. Fish were fed with the same commercial feed once a day (9:00 am) at 1% of body fresh weight. Water samples were collected from the fish tank before and 24 h after feeding to allow fish to digest the ingested feed and excrete waste into the aquatic phase and water to be processed through biofilter system. The nutrient profiles in water samples were analyzed using ion chromatography (IC) and the difference in nutrient concentration before and 24 h after feeding was calculated as daily nutrient release rate.

2.5. Plant/fish growth and biomass measurements

The SPAD value, an index of chlorophyll content per unit leaf area, was measured on three youngest, fully expanded leaves using a portable chlorophyll meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan) at 90 days after transplanting for tomato and basil and 30 days after transplanting for lettuce. Five readings per leaf were taken at the central point of a leaf between the midrib and the leaf margin for lettuce and basil, and the terminal leaflet for tomato and the values were averaged. At harvest plant height was measured from the base of the shoot to the terminal growing point. The number of leaves were determined and the total number of leaves per plant was calculated as the sum of the number of leaves removed during production and at harvest. The fresh mass of leaves was determined immediately at each removal and at harvest. Stem fresh mass was also measured. Fresh weight of shoot parts were calculated by summing the fresh weight of individual parts. Plants were oven-dried (over 72 h at 70°C) and weighed for dry weight.

Days from transplanting to first open flower and fruit harvest were recorded during production. The number of flowers and fruits were counted again at harvest. Fruits were harvested every two days when the fruit was at maturation stage 6 based on USDA Visual Aid TM-L-1 tomato-color standards. The fresh mass of fruits was determined immediately upon harvest, and the total number and weight of fruits per plant was the sum of the number and weight of fruits produced at each harvest. Fruit dry mass was obtained by placing them in a drying oven for 1 h at 100°C followed by at 65°C until the fruits were completely dried.

All dried plant samples were filtered through a 10-mesh sieve after grinding with a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ, USA) and kept in plastic vials for nutrient analysis. Five fish were randomly selected to measure fish fresh weights at the beginning and the end of the study.

2.6. Anion and cation measurements

For anion and cation nutrient analysis of water samples, frozen water samples, which were kept in a –20°C freezer, were thawed at room temperature and centrifuged immediately at 12000 rpm for 10 min, and then liquid supernatants were collected and subjected for cation and anion nutrients measurement. For cation nutrient analysis of dried plant samples (Basta and Tabatabai 1985), each sample was weighed to the nearest 0.100 g and placed in a 20-mL glass vial with three drops of 5% H_2SO_4 in ethanol, then ashed in a muffle furnace at 550 °C for 3 hours. After the process, an 8-mL aliquot of 5 mM HCl was added, vortexed for 10 seconds, heated near boiling (90°C), then vortexed again. Plant samples were centrifuged at 12000 rpm for 10 minutes, and then liquid supernatants were collected. For anion nutrient analysis of dried ground plant samples (Beke and Selles 1993), each sample was weighed to the nearest 0.1 g and placed in a 50-mL centrifuge tube with 0.1 g decolorizing carbon and 13.3 mL Millipore water. Then samples were vortexed for 10 seconds and shaken for 30 minutes. The samples were centrifuged at 12000 rpm for 10 min, and liquid supernatants were collected. After being diluted with distilled deionized water (DD water) to a desirable range, each sample was prepared into an autosampler vial for injection.

Determination and quantification of the nutrient compositions of processed water and plant samples were performed using the ion chromatograph system (Thermo Scientific Dionex ICS–5000, Waltham, US) equipped with capillary pumps, electrolytic eluent generation modules, injection valves, capillary electrochemical suppressors, cation column (IonPac CS12A) and anion column (IonPac AS18 column), and conductivity detectors to determine the concentration of cations (including ammonium, magnesium, calcium, potassium, sodium) and anions (including nitrite, nitrate, phosphate, sulfate, chloride). Flow rates were set at 1 $\mu M \cdot min^{-1}$ and the column temperature was maintained at 20˚C in isocratic mode for cations and gradient mode for anions.

The IC was coupled to an AS–AP autosampler (Thermo Scientific, Waltham, US), allowing for continuous sample loading and injection in sequence including standards and samples. Conditions for IC anion analyses were as follows: eluent (23 mN KOH) flow rate was set at 1 mL min^{-1} and suppressor current was set at 57 mA and raised to 99 mA during gradient runs of the eluent (40 mN KOH), which was conducted for optimal phosphate analysis. A gradient elution method was employed in which eluent concentration was increased from 23 to 40 mM at 12 minutes, remained at 40 mM for 3 minutes, and then decreased to 23 mM for 4 minutes. Conditions for IC cation analyses are as follows: eluent (20 mM MSA) flow rate was set at 1.0 mL min^{-1} for isocratic runs and suppressor current was set at 59 mA. Chromeleon data management software (version 7.1) was used for data processing.

2.7. Total nitrogen and phosphorus measurement

For total nitrogen analysis of each plant sample, 30 mg ground sample was measured and transferred into an empty sample tin using a clean small sampling spatula, then the tin was carefully wrapped up into a ball. The total nitrogen contents of sample was then measured by using the C/N analyzer (FlashEA 1112, Thermo Fisher Scientific, Waltham, MA, USA). When preparing plant samples for total phosphate analysis, each sample was weighed at 0.07 g aliquot and the aliquot weight was recorded before transferred into a 20 mL glass vial. Then the samples were ashed in a muffle furnace at 495 °C for 8 hours. The total phosphate content of each sample was determined

using the P-molybdate blue color reaction (Murphy and Riley 1962) and analyzed by Epoch microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

2.8. Experimental design and data analysis

Each experiment was conducted for 3 months at three different time blocks: spring (December through February), summer (April through June) and autumn (July through September).

Each time block consisted of three aquaponic units (**Figure 1 A**) and three hydroponic units (**Figure 1 B**). The experimental design was a split-plot randomized complete block design (RCBD) with production system and plant species as the main plots: tomato-based aquaponics, basil-based aquaponics, lettuce-based aquaponics, tomato-based hydroponics, basil-based hydroponics, and lettuce-based hydroponics; and with research trial as subplots. The experiment was repeated at three-time blocks and data were pooled across time blocks. Environmental data were presented only for time block 1 (**Figure S1**). Plant growth data were analyzed within each crop. All data were statistically analyzed using JMP v12.0 for Windows software (JMP v23.0 SAS Co. Ltd., USA). Production system and plant species were considered as fixed variables, while season was considered as a random variable. The statistical differences were determined using a two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test or Student's t test ($P \leq 0.05$). The Pearson's correlation coefficient was carried out to determine the association of nutrient (total N, Ca, and Mg) concentrations in the leaves of tomato, basil, and lettuce for the SPAD value.

3. Results

3.1. Nutrient sources and water physical and chemical properties

When nutrient release from the ingested fish feed was quantified, it was found that daily nutrient inputs, as expressed as EC, were nearly 20 times lower in aquaponics compared to that in hydroponics (**Table 2**). Daily nutrient release rates of individual dissolved nutrients were significantly lower than the nutrients contained in hydroponic solutions, except NO_2–N, NH_4–N, Na, and Cl. Despite the low nutrient profile, background nutrients contained in RO water contributed to 30% EC of daily nutrient profiles in aquaponic solutions (**Table 2**). Water parameters, such as dissolved oxygen (DO), water temperature, pH, and EC, were significantly affected by crop species ($P < 0.05$ for DO, pH, and EC; $P < 0.001$ for water temperature) and production system ($P < 0.001$) (**Table 3**). All aforementioned parameters were within optimal ranges for a raft aquaponic system producing tilapia (Al-Hafedh et al. 2008; Danaher et al. 2011; Rakocy 1997). There were significant interactions in water temperature, pH, and EC between crop and system except DO. Regardless of production system, DO and temperature, in fish tank (or nutrient reservoir) remained relatively constant during study period. DO was significantly ($P < 0.0001$) lower but water temperature was significantly higher in aquaponics compared to hydroponics, due to the set temperature difference. Crop species affected water temperature in aquaponics, and higher water temperature was observed in lettuce-based aquaponics compared to tomato- and basil-based aquaponic systems. Similarly, the average pH in aquaponics was higher

than that in hydroponics due to the differences in target pH of each system (**Figure 2**). The pH of nutrient solution was averaged at 6.7 and 5.8 across all crops in aquaponics and hydroponics treatments, respectively (**Table 3**).

Crop species varying in harvest part and method significantly affected EC levels of aquaponic solution over time (**Figure 2**), indicating that nutrient removal capacity, estimated from the changes in EC between 0 and 90 days after transplanting, significantly varied among vegetable crop species. The average EC values in tomato-, basil-, lettuce-based aquaponics were 3.3-, 2.1-, 1.9-times lower than those in hydroponics, respectively (**Table 3**). Especially during the first month when the systems were young, the EC levels were significantly lower in aquaponics than in hydroponics, but gradually increased over a 3-month production period (**Figure 2**). Particularly, the EC level of lettuce-based aquaponics linearly increased and reached nearly to the level of hydroponics after being operated for 3 months. Meanwhile, the EC levels of tomato- and basil-based aquaponics remained at 0.6 and 1.2 ds m^{-1}, respectively, during the third month (**Figure 2**).

3.2. Growth and yield of vegetables and fish

Overall, aquaponics significantly reduced vegetative shoot growth of cherry tomato, basil, and lettuce compared to those grown in hydroponics (**Table 4**). For example, tomato grown in aquaponics decreased plant height and leaf length than those grown in hydroponics, and basil showed a decreasing tendency of these growth parameters in aquaponics than those grown in hydroponics. In contract, there was no significant difference in lettuce growth between the systems. Likewise, leaves of tomato plants grown in aquaponics had significantly lower SPAD values than those in hydroponics, and basil had a tendency of lower SPAD values when grown in aquaponics (**Table 5**). In contrast, SPAD values of lettuce were not affected by production systems.

Crop yield including total fresh and dry mass were highly influenced by production system ($P < 0.0001$) and crop species ($P < 0.0001$) (**Table 5**). Regardless of plant species, vegetative shoots were markedly reduced in aquaponics, with more pronounced reduction in tomato shoots by more than 50%. However, marketable yield, i.e., the number of fruits and fruit yield, of tomato grown in aquaponics was not significantly different from that in hydroponics (**Tables 4 and 5**). Marketable yields of basil and lettuce (i.e., shoot fresh mass) grown in aquaponics were reduced by 44% and 33%, respectively, than those in hydroponics. Meanwhile, yield of lettuce was significantly increased with harvest time (**Table 6**), which coincided with maturity of the system. Lettuce yield in aquaponics was 43% of that in hydroponics at the first harvest, but increased to 76% at the third harvest. Root-to-shoot ratio was significantly higher in basil and lettuce grown in aquaponics, indicating proportional increase of roots in these vegetable crops (**Table 5**).

Although the same amount of fish feed was applied, the total biomass increment rate of tilapia fish tended to be higher in tomato-based aquaponics, followed by basil- and lettuce-based systems (**Table 3**). Cumulative water consumption was significantly ($P < 0.05$) higher in aquaponics; however, it was not affected by vegetable crops.

3.3. Nutrient concentrations and accumulations in recirculating water

Aquaponics had significantly (P < 0.0001) lower average concentrations of NO_3–N, PO_4–P, K, Ca, Mg, and SO_4–S in recirculating water but significantly (P < 0.0001) higher concentrations of NO_2–N, NH_4–N, Na, and Cl compared to those in hydroponics (**Table 7**). Especially, the average concentrations of Ca and Mg in aquaponics were 8- and 25-times, respectively, lower than those in hydroponics. Aquaponic solution contained the following nutrients in decreasing order: SO_4–S (242 mg L^{-1}) > NO_3–N (110 mg L^{-1}) > K (75 mg L^{-1}) > Na (70 mg L^{-1}) > PO_4–P (30 mg L^{-1}) > Ca (18 mg L^{-1}) > Cl (4.3 mg L^{-1}) > Mg (1.6 mg L^{-1}) (**Table 7**). Meanwhile, hydroponic solution contained the following nutrients in descending order: SO_4–S (677 mg L^{-1}) > K (333 mg L^{-1}) > NO_3–N (200 mg L^{-1}) > Ca (146 mg L^{-1}) > PO_4–P (119 mg L^{-1}) > Mg (40 mg L^{-1}) > Na (11 mg L^{-1}).

There were interactions between production system and crop species in NO_3–N, PO_4–P, Ca, Mg, and Cl levels (**Table 7**). With exception of PO_4–P, the levels of these nutrients in aquatic phase of the system were significantly affected by crop type (P < 0.001), indicating that tomato removes more NO_3–N, Ca, Mg, and Cl from recirculating water than basil and lettuce, but there were no differences in removing PO_4–P from the water among the plants examined in this study. In general, the levels of aforementioned nutrient elements, especially NO_3–N, were the highest in lettuce-based aquaponics followed by basil- and tomato-based systems, while there were no significant differences in NO_3–N levels in hydroponic solutions regardless of plant species.

Nutrient accumulation rate in aquatic phase was significantly higher in lettuce-based system, followed by basil- and tomato-based systems (**Figure 3**). The concentrations of mineral nutrients in both systems showed an increasing or decreasing trend during production period depending on the system and nutrient (**Figure 3**). In general, the concentrations of nutrients gradually increased over time in lettuce-based aquaponics while it decreased in tomato-based aquaponics (**Figure 3**). For example, NO_3–N concentrations in lettuce-based aquaponics increased from 90 to 250 mg L^{-1} during a 3-month production, and even exceeded those in hydroponics at the third month (**Figure 3 A**). Contrarily, the initial concentrations of NO_2–N and NH_4–N were 14 and 65 mg L^{-1} but the levels decreased rapidly and were nearly 0 mg L^{-1} as the system grew mature (**Figure 3 B, C**), indicating active nitrification taking place in aquaponic systems. Meanwhile, PO_4–P concentrations averaged at 30 mg L^{-1} regardless of crop species while fluctuating in association with crop harvest, and showed increasing trends of concentrations in all vegetable crops (**Figure 3 D**).

The concentrations of all nutrients were highly accumulated in lettuce-based hydroponics throughout production period, primarily due to high initial nutrient concentrations used in the systems (**Table 2**; **Figure 3**). For example, the NO_3–N concentrations gradually increased from 170 to 220 mg L^{-1} in hydroponic systems. Similarly to aquaponic systems, nutrient accumulation in aquatic phase was significantly increased in lettuce-based hydroponics, followed by basil- and tomato-based systems. In general, the concentrations of nutrient elements gradually increased over time in all hydroponic systems (**Figure 4-2**), although the EC levels were constantly maintained at 2 dS m^{-2}. Particularly, PO_4–P levels were highly maintained and averaged at 120 mg L^{-1}, which was 4-times higher than those in aquaponics (**Figure 3 D**; **Table 7**).

3.4. Nutrient concentrations and accumulations in plant tissues

Nutrient accumulation in plant tissues was significantly (P < 0.0001) affected by production system, crop species, and tissue type (**Table 8**). Regardless of crop species, vegetable crops grown in aquaponics accumulated lower levels of N, P, Ca, Mg, and S in plant tissues than those in hydroponics, but accumulated higher levels of Na and Cl. Meanwhile, there were no significant differences in K accumulation levels between the systems. Crop species also demonstrated different nutrient accumulation capacity: lettuce accumulated the highest levels of N, P, K, Na, and Cl in mg g^{-1} on a dry matter basis in comparison to tomato. In general, leaves were the major plant parts where the highest concentration of nutrients were accumulated with exception of P and S, which mainly accumulated in roots and stems, respectively.

Figure 4 showed nutrient accumulation patterns of tomato, basil, and lettuce grown in aquaponics and hydroponic systems in fruits, leaves, stems, and roots. Vegetable crops grown in aquaponic systems had reduced accumulation of total N in above-ground parts (**Figure 4-1**). However, vegetable crops in aquaponics had increased accumulation of Ca and Na in fruits of tomato, and above-ground shoots (either leaves or stems) of basil and lettuce (**Figure 4-2**), although Ca levels in aquaponic solution was significantly lower than in hydroponic solution (**Table 7**; **Figure 3**). Na accumulation levels varied greatly by plant species and plant parts and were approximately 2-times higher in edible parts of plants grown in aquaponics than those in hydroponics (**Figure 4-2**).

4．Discussion

4.1. Water quality parameters

Water quality parameters directly impact fish health and wellbeing and plant growth in aquaponics, and therefore, are the primary considerations that should be optimized for the improvement of aquaponic production and yield. The deterioration of water quality parameters affects fish physiology, growth rate, and feed efficiency, leading to pathological changes and even mortality under extreme conditions (Yildiz et al. 2017). Dissolved oxygen (DO) concentration was decreased by 24% in aquaponics compared to hydroponics regardless of crop species due to the oxygen demands of additional organisms (i.e., fish and microbes) in the system. Despite, DO in aquaponics averaged at 7 mg L^{-1}, which was well above the tolerance limits of 6 mg L^{-1} (Graber and Junge 2009) and 30% higher than 5 mg L^{-1}, suggested DO level for aquaculture (Boyd 1982). In fact, the DO levels in our system were over 85% saturated at water temperature of 26 to 28ºC, which were suitable for plant and fish growth and nitrification process as evidenced by rapid conversion of NH$_4$–N to NO$_3$–N (**Figure 3 A, B, and C**).

Water temperature was higher in aquaponics than hydroponics (**Table 3**) due to the use of warmer-water for tilapia culture. However, water temperature in aquaponics was higher in lettuce-based aquaponics than tomato- and basil-based systems in all three time blocks. Although the cause is not clearly understood, water characteristics in lettuce-based aquaponics might have set the biological limits for sustainable production, affecting water temperature. In other words, unstable/poor water conditions in lettuce-based aquaponic system, relative to tomato- and basil-

based systems, might have caused impaired biological capacity of the fish, affecting welfare conditions through complex interactions between water quality parameters (Yildiz et al. 2017). This view can be supported by a lower trend of fish biomass increment in lettuce-based aquaponics compared to other aquaponic systems (**Table 3**) despite the fact that the same amount of fish feed was applied.

The optimal pH ranges for each organism are varied (6 to 9 for tilapia fish, 5.5 to 6 for plants, and 7 to 8 for nitrifying bacteria), and therefore, the trade-off pH for aquaponics is considered to 7 (Rakocy et al. 2006). The pH from 6 to 8 is optimal for the uptake of macronutrients while micronutrients are preferentially absorbed at pH levels below 6 (Marschner 2012). While such high pH level is likely to compromise plant growth due to limited availability of micronutrients, those were not considered in this study due to relatively low demands of plants (Marschner 2012). Our results demonstrated that all macronutrients are available at this target pH range, but supplemental nutrients may be needed depending on the type of vegetable crops in the system (i.e., Ca and Mg for tomato; Mg for basil), due to the high demands relative to low supply. This aspect will be discussed further.

4.2. Crop growth and yield

Overall, fresh and dry mass production of tomato plants significantly decreased in aquaponics compared to hydroponics (**Table 5**). In fact, such reduced vegetative growth accelerated flowering and fruiting of tomato plants grown in aquaponics (data not shown), without affecting fruit yield (**Table 5**). It has been reported that reduced biomass allocation to vegetative parts can promote early flowering and reproductive growth as demonstrated in greenhouse hydroponic tomato (Kim et al. 2018a). Reduced nutrient levels in aquaponics might have promoted transition from vegetative to reproductive growth of tomato plants (Marschner 2012). Such growth responses would make it easier for crop management in aquaponics by producing smaller above-ground biomass without compromising crop yield.

Similarly, fresh and dry mass production of basil and lettuce decreased or had a decreasing tendency in aquaponics as compared to hydroponics. The EC level is likely to be one of the critical factors for lettuce yield, because lettuce yield increased by 2-times with increasing average EC from 0.37 to 1.44 dS m^{-1} (**Table 6**; **Figure 2**). However, lettuce yield was consistently lower in aquaponics even when the EC was nearly 70% of that in hydroponics at the third month (**Table 6**), in which the average NO$_3$–N level (222 mg L^{-1}) in aquaponic solution was even higher than that in hydroponic solution (204 mg L^{-1}), indicating that NO$_3$–N is not a single factor affecting crop yield in aquaponics. These results indicate that reduced crop yield in aquaponics is likely to be related to the combinational effects of water chemical properties including pH, EC, and nutrient compositions (i.e., lower NO$_3$–N, Ca, and Mg, and higher NO$_2$–N, NH$_4$–N, Na), in addition to water biological properties which were not investigated in this study.

It has been considered that reduced yield in aquaponics is mainly associated with low availability of K, Ca, P and micronutrients such as Fe and Mn (Rakocy et al. 2004; Roosta 2014). Therefore, it has been suggested to consider nutrient supplements (Rakocy et al. 2004; Rakocy et al. 2006), foliar application of fertilizer (Roosta and Hamidpour 2011), or inoculation with phosphorus solubilizing microorganisms (da Silva and Fitzsimmons 2016) to improve crop yield. For example,

tomato plants grown in aquaponics had as high yield as those in conventional hydroponics when applied with foliar application of K > Fe > Mn > Zn > Mg > B in decreasing order of effectiveness (Roosta and Hamidpour 2011). Nutrient supplements in aquaponic solution with either Fe or Fe plus macronutrients (P, K, Mg, and S) increased lettuce growth and yield (Nozzi et al. 2018). We found that tomato plants grown in aquaponics produced a similar fruit yield as hydroponics even without nutrient supplements or foliar spray with nutrients (**Table 5**). Likewise, total fresh and dry mass of lettuce was not different between aquaponics and hydroponics without any nutrient application. In fact, we identified key macronutrient elements that are deficient in aquaponics solution and need to be supplemented for the improvement of crop growth and yield. This can be varied depending on the type of water used, which will be discussed in the following section.

During a 3-month production of tomato, basil, and lettuce, aquaponics tended to use 22%, 34%, and 45% more water than hydroponics. Cumulative water use of a tomato-based aquaponic system was not significantly different from those of basil- and lettuce-based systems, despite its higher biomass gains of vegetables and fish (**Tables 3 and 5**). Given that EC and nutrient profiles were constantly low in tomato-based aquaponics, relatively higher trend of fish mass gain in tomato-based aquaponics may be attributed to better water quality associated with higher nutrient removal of tomato plants.

4.3. Nutrient accumulation in recirculating water and plant tissue

Considering the nature of recirculating aquaponic systems, i.e., daily nutrient inputs and minimal water inputs, it is well expected that dissolved nutrients accumulate over time in recirculating water. EC levels in recirculating aquaponics increased linearly when plants were relatively young, and showed different increasing patterns depending on the type of vegetable crops, with nearly 10-, 18-, and 34-times increases in tomato-, basil-, and lettuce-based systems, respectively, over a 3-month production period (**Figure 2**). It was clear that fish feed (41% protein; 1.1% P) was provided beyond nutritional needs of fish and uptake rate of plants. Entire lettuce plants were harvested from the system at maturity, while the roots of tomato and basil were kept intact actively removing nutrients. The significant difference in EC levels found from the second month indicate the variation in nutrient removal capacity among vegetable species partly due to different harvest methods and parts.

Aquaponics not only had lower initial concentrations of nutrients but also had considerably lower daily nutrient release rate than that in hydroponics (**Table 2**). Such differences in nutrient environment as well as other water chemical properties, especially at the initial stage, possibly affected transplant establishment, subsequently affecting plant growth and development as demonstrated in our study. Although daily nutrient release rate appeared to be a small contribution to nutrient profiles, it had considerable effects on increasing EC levels over time in all aquaponic systems, particularly in lettuce-based aquaponics (**Figure 2**). Our results demonstrated that the EC levels in aquaponics was contributed by three sources: primarily by daily nutrient release from fish feed ingested, and secondarily by background nutrients contained in RO water and the solutions added for pH corrections, as the volumes needed for pH correction in aquaponics were over 5-fold higher than those in hydroponics due to the active nitrification in the system. These practices promoted the steady accumulation of SO_4–S > NO_3–N > K > Na > PO_4–P > Ca > Cl > Mg in decreasing order of nutrient elements in aquaponic solution. While EC was maintained at nearly 2

dS m^{-1} in hydroponics, daily nutrient replenishment and the changes in plant uptake patterns of an individual nutrients gradually also altered the compositions of hydroponic solutions (**Figure 3**). Notably, despite the lower daily nutrient inputs compared to hydroponics and continued removal of nutrients through biomass production, most of macronutrients showed increasing trend of accumulation in aquaponics (**Figure 3**), justifying the need for proper nutrient management. Macronutrients, i.e., N, P, K, Ca, Mg, and S, are required in relatively large quantities (> 0.1% of dry mass) and essential for plants to complete their life cycle (Maathuis 2009; Marschner 2012). These mineral nutrients are taken up by plant roots in ionic forms: N as anionic nitrate (NO_3–N) or cation ammonium (NH_4–N), and P and S as their oxyanions phosphate (PO_4–P) and sulfate (SO_4–S), and K, Ca, and Mg as free cations (Maathuis 2009). Liebig's law of the minimum states that yield is proportional to the amount of the most limiting nutrient. Thus, we postulated that some of these macronutrients are a major factor limiting plant growth in aquaponics, due to an insufficient level released from fish feed.

4.3.1. Concentrations of Ca and Mg derived from fish feed were critically low
In addition to the differences in initial concentrations of Ca and Mg, daily release rates of Ca and Mg in aquaponics were only 4 to 10% and 1.5 to 2.5%, respectively, of the daily replenishment rates of those in hydroponics (**Table 2**). This explains the critical differences in concentrations of these elements between aquaponic and hydroponic solutions (**Table 7**; **Figure 3**). Accordingly, leaves and stems of tomato grown in aquaponics had significantly lower Ca and Mg concentrations (**Table 8**; **Figure 4-2 E, F**). Therefore, leaf chlorosis observed in tomato grown in aquaponics is highly likely to be the combinational effects of insufficient Ca and Mg. The levels of these ions in tomato- and basil-based aquaponics rapidly declined especially during active vegetative growth and fruit development (**Figure 3**), indicating the needs of supplemental Ca and Mg during this period. Such low levels of Ca and Mg in aquaponics solution were associated with significantly lower SPAD values in the leaves of tomato (Ca, P < 0.01; Mg, P < 0.05) and basil (Mg, P < 0.12) (**Table S2**). On the contrary, leaves of lettuce grown in aquaponics had higher Ca concentration than those in hydroponics. In fact, 100% of the lettuce grown in hydroponics developed Ca deficiency in young leaves (a condition known as tip-burn) in the fourth week after transplanting, which was not observed in lettuce grown in aquaponics. The balance between plant growth rate and nutrient uptake rate can explain much of these contrasting results (Halevy 1976). Growth rate of lettuce was higher in hydroponics than aquaponics (data not shown) possibly due to luxuriant nutrients, exceeding the nutrient uptake rate of Ca. Such plant response can be also varied by the type of aquaponic system because Ca and Mg was not of concern in gravel-based aquaponics (Seawright et al. 1998) or in aquaponics using water source containing high levels of Ca and Mg (Goddek et al. 2016). SPAD value was also strongly correlated with leaf total N content in tomato ($r^2 = 0.82$, P < 0.001), basil ($r^2 = 0.80$, P < 0.01), and lettuce ($r^2 = 0.59$, P < 0.05) (**Table S2**). Multiple regression analysis supported our interpretation that the combined effects of total N, Ca, and Mg concentrations in the leaves contributed to the leaf greenness (SPAD value) of tomato, basil, and lettuce, rather than the individual nutrient effects (**Table S2**).

Similarly to our results, lettuce leaves contained significantly lower Ca when grown in tilapia-based aquaponics (RO water was used) than in hydroponics (Pantanella et al. 2012). Lettuce grown in aquaponics had low Ca and Mg (rainwater was used; nutrient solution was formulated by

diluting recirculating aquaculture system water and adding mineral salts) (Delaide et al. 2016), although this aspect was not addressed by the authors. These results indicate that Ca and Mg are the major limiting factors of crop growth in aquaponics, especially when water containing little Ca and Mg is used, such as RO water, distilled water, and rainwater, and possibly other type of water depending on the source and region (Azoulay et al. 2001; Pehrsson et al. 2008). In fact, Ca and Mg deficiency have not been paid much attention in aquaponic studies so far, probably due to common use of water sources containing high concentrations of Ca and Mg. However, lack of these nutrient elements can lead to negative impacts on crop performance as demonstrated in our study.

4.3.2. Na source and accumulation in aquaponics
Our results showed that the levels of Na were 5- to 7-times higher in aquaponics compared to hydroponics. Na toxicity has been suggested as one of the limiting factors in aquaponic systems. Although the source of Na was not clear, it has been considered either from fish feed or solutions used for pH correction (Seawright et al. 1998; Delaide et al. 2016). Considering that both pH correction solution and RO water containing Na contributed to steady a level of Na in hydroponic solution (**Figure 3 I**), Na sources in aquaponics are likely to be fish feed, pH correction solution, and water used in the system. Daily release rate of Na was only 0.4% of fish feed applied; however, it accumulated rapidly in recirculating water during the second month (**Table 2**; **Figure 3 I**). This might have caused not only by NaOH for pH adjustment, but more importantly by background Na contained in RO water because more frequent replenishment and higher volume of water was required in aquaponics (**Table 3**). Consequently, such high level of Na in aquaponic solution resulted in a 3-fold higher Na concentration in the tissues of plants grown in aquaponics than those in hydroponics (**Table 8**). Daily Na release rate from fish feed was significantly less compared to Na accumulation rate in aquaponics during the second month (**Table 2**; **Figure 3**), indicating that the changes in Na accumulation in water may depend on the age and size of fish and plants.

In our study, Na was more rapidly accumulated in lettuce-based aquaponics than basil- and tomato-based systems. It is well recognized that salinity disrupts mineral relations of plants by reducing nutrient availability leading to Na-induced Ca and/or K deficiencies and Ca-induced Mg deficiencies (Grattan and Grieve 1992). As such, high concentration of Na is commonly known to cause negative impact on crop yield; however, this appears to be concentration-dependent. For example, irrigation with high Na concentration (i.e., > 100 mM; 2300 mg L^{-1}) for 15 days significantly reduced yield of baby romaine lettuce, while irrigation with a relatively low salt concentration (i.e., 5 mM; 115 mg L^{-1}) did not reduce its growth and appearance (Kim et al. 2008b). In our study, Na concentration increased to 110 mg L^{-1} in aquaponic systems as the system reached to maturity, which did not cause any detrimental effects on plant growth and yield but significantly reduced shoot biomass of tomato, basil, and lettuce. It is not clear if the Na concentration in aquaponic solution is one of the direct causes of reduced shoot biomass. This aspect needs further investigation.

4.3.3. N accumulation in aquaponics
Lettuce is a well-known nitrate accumulating plant (Colla et al. 2018). In fact, we found that lettuce accumulates higher N, P, Na, and Cl in plant tissues relative to tomato and basil (**Table 8**).

However, we observed faster NO_3–N accumulation in lettuce-based aquaponics compared to tomato- and basil-based ones. The results indicate that N transformation rate from NH_3–N to NO_3–N mediated by nitrifying bacteria outpaced the NO_3–N removal rate of plants in our system and that harvest practice of lettuce encourages NO_3–N accumulation in aquaponic solution. Considering the linear increase of NO_3–N, particularly in lettuce-based aquaponics, nitrate accumulation in the edible parts of vegetables may be expected when growing vegetables in mature aquaponic systems. Therefore, long-term production of vegetable crops in aquaponics may negatively impact crop production and quality. In contrast to basil- and lettuce-based aquaponics, NO_3–N gradually declined in tomato-based system (**Figure 3**). These results indicate that NO_3–N uptake rate of tomato exceeded its release rate derived from fish feed and that tomato plants continued to actively remove NO_3–N from aquaponic solution to support the growth of developing fruits and vegetative parts. Contrarily, the initial sharp decline of NO_2–N and NH_4–N was observed in aquaponics which can be accounted for by the active nitrification. Aquaponics water contained higher levels of toxic ions (NO_2–N and NH_4–N) than hydroponics, and such unique chemical properties may be one of the causes for reduced growth and biomass production of tomato, basil, and lettuce grown in aquaponics (**Tables 5 and 6**).

4.3.4. P accumulation in aquaponics

Many aquaponics studies have demonstrated an initial sharp decline of P concentrations (Seawright et al. 1998) or low P concentrations maintained in recirculating water of aquaponics (Goddek et al. 2015; Rakocy et al. 2004). Typically, P level ranged from 1 to 7 mg L^{-1} in most aquaponics studies. Precipitation of P was considered for such low P levels in aquaponics, which is often associated with high availability of Ca and Mg (Goddek et al. 2015; Savidov et al. 2007; Seawright et al. 1998). In fact, aquaponic studies have used various sources of water: well water and rainwater (Rakocy et al. 2004), fresh water (Lennard and Leonard 2006), groundwater and rainwater (Rakocy et al. 2007), tap water (Goddek et al. 2016; Delaide et al. 2017), or rainwater (Al-Hafedh et al. 2008), in which high Ca and Mg levels in aquaponic solution have been reported. However, our results showed that daily P release rate in aquaponics was 26% of ingested fish feed, gradullay increasing dissolved P in water at an average concentration of 30 mg L^{-1} (**Table 2**; **Figure 3 D**). Dissolved P derived from fish feed containing 1.1% P is considered sufficient to support plant growth. This judgement can be further supported by fact that total P levels in plant tissues of tomato, basil and lettuce grown in aquaponics were similar or slightly lower than those grown in hydroponics (**Figure 4-1**). Consistently, Pantanella et al. (2012) reported that P level increased from 3 up to 30 mg L^{-1} after 4 week production of romain lettuce in aquaponics filled with RO water. These collective information indicates that P is not a limiting factor in aquaponics and water source may be the key in determining P availability in aquaponic systems. Low P availability in aquaponics may be in part due to the use of plant-based formulations containing high amounts of phytic acid (or phytate), the major storage form of phosphorus in plant tissues. The addition of phytase or inoculation with Bacillus spp. was suggested to be effective in liberating free phosphate from plant-based formulations (da Silva and Fitzsimmons 2016).

P application rate can be as high as 20 mg L^{-1} in soil-based (Santos et al. 2004) and substrate-based (inert substrates were used) production systems (Kim and Li 2016), impying that P levels can be

considerably lowered in water-based systems. In a typical soil environment, P is limited by soil depth, and P acquisition is determined by root architectural traits (Kim et al. 2008a). However, in water-based systems including aquaponics, P exists mainly in two forms, dissolved and particulate forms, and only phosphates, dissolved form, can be bioavailable for plant to uptake and remove from the recirculating water, where diffusion gradients are not an issue as long as P is supplied to root zone at a constant rate. Similar observations have been made previously in other plants for NO_3–N with a minimum concentration threshold of 1 mg L^{-1} (Clement et al. 1978; Edwards and Barber 1976; Warncke and Barber 1974). There were no significant differences on average shoot and root fresh weight of Romaine lettuce cultivated in deep-water culture for 26 days with a wide range of NO_3–N concentrations from 5 to 105 mg L^{-1} (Letey et al. 1982). Given that most of nutrients accumulated in aquaponic solution, including about 26% ionic phosphate released from fish feed, it would be desirable to utilize fish feed containing less protein and P for aquaponics to improve efficiency of the system.

5. Conclusions

In this study, we characterized nutrient profiles in aquaponics and identified key nutrient elements affecting crop growth and yield. Our direct comparisons with hydroponic system enabled us to determine nutrients that are deficient or in excess in aquaponic solution and plant tissues. Despite relatively low daily nutrient release rate from ingested fish feed, most nutrients gradually accumulated in aquaponic solution and consequently in plant tissues. Aquaponics reduced vegetative shoot growth of all tested crops; however, fruit yield was not affected in tomato by the reduced growth. We found that N, Ca and/or Mg were temporarily suboptimal in aquaponics during crop production, reducing crop growth and yield, while P, Na, and Cl were maintained at sufficient or supraoptimal levels. Nutrient management should be established by considering these dynamic changes in nutrient elements to improve crop production in aquaponic systems. Our results indicate that luxuriant nutrient profiles in hydroponics are not necessary to enhance crop yield in aquaponics as long as key factors affecting crop yield are identified and properly addressed. Further, aquaponic nutrient management should be considered in accordance with water source and supplement target nutrients to enhance crop growth and yield and improve efficiency of the system. Our results will aid in establishing production guidelines and quality standards for the expansion of commercial aquaponics.

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Table 2. Composition and concentration of nutrient sources used in tomato-, basil-, and lettuce-based aquaponics and hydroponics.

Macronutrient	Aquaponics	Hydroponics					RO water[e] (mg L^{-1})
	Daily release rate[a] (mg L^{-1} day^{-1})	Daily replenishment rate[c] (mg L^{-1} day^{-1})			Initial concentration[d] (mg L^{-1})		
		Tomato	Basil	Lettuce	Tomato	Basil/Lettuce	
NO$_3$–N	1.04 (1.6%)[b]	9.29	5.00	4.52	178.7	161.4	0.42
NO$_2$–N	0.13 (0.2%)	0.37	0.16	0.14	7.1	5.1	–
NH$_4$–N	0.40 (0.6%)	1.15	0.44	0.39	22.2	14.1	0.02
PO$_4$–P	2.41 (25.7%)	4.92	2.80	2.53	94.6	90.2	0.44
K	0.82 (7.8%)	8.41	5.59	5.05	161.7	180.3	0.34
SO$_4$–S	15.7 (35.3%)	21.5	14.6	13.2	413.1	471.7	1.24
Ca	0.20 (0.7%)	5.39	3.53	3.19	103.7	113.9	2.45
Mg	0.02 (0.9%)	1.38	0.90	0.81	26.6	29.0	0.59
Na	0.11 (0.4%)	–	–	–	–	–	2.76
Cl	0.50 (2.2%)	–	–	–	–	–	2.30
EC (dS m^{-1})	0.1				2.0	2.0	0.03
pH	6.9				6.0	6.0	7.3

Each value in the table is the mean of 15 replicates for aquaponics and 6 replicates for hydroponics.

[a] Nutrient concentrations (mg L^{-1} day^{-1}) in aquatic phase of an aquaponics system released daily when 1% fish feed (41% protein; 1.1% phosphorus) was applied to tilapia fish (average fish weight: 250 g; stocking density: 20 kg m^{-3}). Nutrient release was monitored in the absence of plants and average daily release rate was presented here.

[b] Daily nutrient release rate (%) from fish feed applied (100%).

[c] Nutrient concentrations of commercial fertilizer in hydroponic nutrient solution. Fertilizer was dissolved in reverse osmosis water at 1:100 dilution and electrical conductivity (EC) was set at 2 dS m^{-1}.

[d] Initial nutrient concentrations in hydroponic solution.

[e] Background nutrient concentrations contained in reverse osmosis (RO) water.

Table 3. Average water quality parameters, fish biomass increment, and cumulative water use during 3-month production of tomato, basil, or lettuce-based aquaponics and hydroponics.

Crop	Production system	DO (mg L^{-1})	Water temperature (°C)	pH	EC (dS m^{-1})	Fish biomass increment[a] (%)	Cumulative water use[b] (L)
Tomato	Aquaponics	7.21 ± 0.09^b	26.9 ± 0.10^b	6.88 ± 0.02^a	0.54 ± 0.04^c	38.6	465.5 ± 47.5^a
	Hydroponics	9.37 ± 0.06^a	22.1 ± 0.11^c	5.82 ± 0.02^b	1.95 ± 0.02^a	–	365.8 ± 39.6^a
Basil	Aquaponics	7.04 ± 0.08^b	26.6 ± 0.10^b	6.76 ± 0.02^a	0.84 ± 0.09^b	32.5	418.0 ± 19.0^a
	Hydroponics	9.32 ± 0.06^a	22.1 ± 0.13^c	5.83 ± 0.01^b	1.94 ± 0.04^a	–	276.4 ± 95.5^a
Lettuce	Aquaponics	7.11 ± 0.07^b	27.5 ± 0.08^a	6.81 ± 0.02^a	0.92 ± 0.11^b	27.1	437.0 ± 114.0^a
	Hydroponics	9.26 ± 0.06^a	22.2 ± 0.11^c	5.84 ± 0.01^b	1.96 ± 0.04^a	–	239.0 ± 35.5^a
Analysis of variance							
Crop		*	***	*	*	ns	ns
System		***	***	***	***	–	*
Crop × System		ns	***	**	*	–	ns

[a] Feed biomass increment calculated as wet weight gain (final weight – initial weight) × 100 over three months.

[b] Average cumulative water use was calculated based on a 3-month study conducted at three different time blocks.

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean ± SE of 3 replicates.

ns,*,**,*** mean no significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Table 4. Plant height, leaf length and number and fruit number of tomato, basil, and lettuce grown in aquaponics or hydroponics.

Crop	Production system	Plant height (cm)	Leaf length (cm)	Leaf number (n plant^{-1})	Fruit number (n plant^{-1})	SPAD	Plant water content (%)
Tomato	Aquaponics	87.2 ± 6.2^b	39.5 ± 3.9^b	21.0 ± 3.4^c	61.4 ± 4.3^a	24.5 ± 0.4^c	91.7 ± 0.2^c
	Hydroponics	103.7 ±10.8^a	46.3 ± 4.8^a	21.6 ± 3.4^c	51.4 ± 5.6^a	38.6 ± 1.1^a	91.1 ± 0.2^{cd}
Basil	Aquaponics	39.9 ± 1.8^c	11.4 ± 0.3^d	118.3 ±10.2^b	–	23.2 ± 0.9^c	90.7 ± 0.1^d
	Hydroponics	49.8 ± 2.6^c	12.9 ± 0.6^d	140.7 ± 8.2^a	–	31.7 ± 0.4^b	90.9 ± 0.1^d
Lettuce	Aquaponics	16.3 ± 0.2^d	20.5 ± 0.3^c	16.7 ± 0.2^c	–	24.6 ± 0.6^c	96.1 ± 0.1^b
	Hydroponics	18.1 ± 0.2^d	22.1 ± 0.4^c	18.1± 0.4^c	–	25.7 ± 0.6^c	96.5 ± 0.1^a
Analysis of variance		***	***	***	–	***	***
Crop		**	***	ns	ns	***	***
System		ns	*	ns	–	***	ns
Crop × System		***	***	***	–	***	*

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean ± SE of 9 replicates for tomato and basil, and 27 replicates for lettuce.

ns, *, **, *** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5. Crop yield of tomato, basil, and lettuce grown in either an aquaponic or hydroponic system.

Crop	Production system	Fresh mass (g plant^{-1})				Dry mass (g plant^{-1})				Root-to-shoot ratio[a]
		Total	Shoots	Roots	Fruits	Total	Shoots	Roots	Fruits	
Tomato	Aquaponics	1318.5 b	601.7 b	316.9 a	399.9 a	104.7 b	66.2 b	13.8 b	24.8 a	0.18 ab
	Hydroponics	2032.8 a	1300.7 a	337.0 a	395.1 a	179.5 a	130.8 a	23.2 a	25.5 a	0.17 ab
Basil	Aquaponics	306.3 d	213.9 de	92.4 c	–	29.8 d	25.7 d	4.1 d	–	0.19 a
	Hydroponics	545.9 c	385.2 c	160.7 b	–	52.4 c	46.6 c	5.8 c	–	0.15 b
Lettuce	Aquaponics	181.0 d	152.1 e	29.0 d	–	7.0 e	5.8 e	1.2 e	–	0.21 a
	Hydroponics	263.5 d	228.3 d	35.2 d	–	9.3 e	8.2 e	1.1 e	–	0.14 b
Analysis of variance										
Crop		***	***	***	–	***	***	***	–	ns
System		***	***	***	ns	***	***	***	ns	***
Crop × System		***	***	***	–	***	***	***	–	***

[a] The ratios were calculated by g g^{-1} on a dry matter basis.

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean ± SE of 9 replicates for tomato and basil, and 27 replicates for lettuce.

ns, *, **, *** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 6. Yields of lettuce in aquaponics and hydroponics at three harvest times.

Harvest time	System	Marketable yield (g plant^{-1})	Root fresh mass (g plant^{-1})	Total yield (g plant^{-1})
First	Aquaponics	89.7 ± 6.9^c	18.2 ± 1.1^d	107.9 ± 7.5^c
	Hydroponics	210.3 ± 11.1^a	31.2 ± 0.9^b	241.5 ± 11.7^a
Second	Aquaponics	97.1 ± 4.4^c	26.4 ± 0.4^c	123.5 ± 4.7^c
	Hydroponics	148.4 ± 5.6^b	26.1 ± 0.5^c	174.5 ± 5.9^b
Third	Aquaponics	177.3 ± 7.2^b	26.9 ± 0.3^c	204.2 ± 7.4^b
	Hydroponics	233.6 ± 10.0^a	37.0 ± 1.0^a	270.6 ± 10.8^a
Analysis of variance				
Time		***	***	***
System		***	***	***
Time × System		ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean ± SE of 9 replicates for lettuce.

ns, *, **, *** mean no significant or significant at $P ≤ 0.05$, 0.01, or 0.001, respectively.

Table 7. Average mineral nutrient concentrations in the aquatic phase of tomato-, basil- or lettuce-based aquaponics and hydroponics during four-month production period.

Crop	Production system	Mineral nutrient (mg L⁻¹)									
		NO$_3$–N	NO$_2$–N	NH$_4$–N	PO$_4$–P	K	Ca	Mg	SO$_4$–S	Na	Cl
Tomato	Aquaponics	60.5 d	4.4 a	1.8 a	30.1 c	29.2 c	12.1 d	0.6 d	218.1 b	61.9 a	0.0 c
	Hydroponics	207.9 a	0.07 b	0.4 bc	122.8 ab	334.0 a	141.9 b	39.4 ab	627.5 a	8.6 b	0.0 c
Basil	Aquaponics	108.0 c	5.0 a	1.7 ab	33.0 c	82.3 b	22.6 c	1.9 c	267.8 b	76.7 a	1.7 b
	Hydroponics	193.8 a	0.1 b	0.3 bc	107.4 b	326.8 a	145.8 ab	40.5 a	674.5 a	14.0 b	0.0 c
Lettuce	Aquaponics	161.6 b	4.8 a	1.8 a	27.1 c	114.1 b	20.4 c	2.4 c	235.8 b	70.0 a	11.2 a
	Hydroponics	198.6 a	0.05 b	0.2 c	125.5 a	339.0 a	148.8 a	39.0 b	727.8 a	10.7 b	0.0 c
Analysis of variance											
System		***	***	***	***	***	***	***	***	***	***
Crop		***	ns	ns	ns	ns	***	**	ns	ns	***
System × Crop		***	ns	ns	**	ns	***	***	ns	ns	***
System											
AQU		110.0 b	4.7 a	1.8 a	30.1 b	75.2 b	18.4 b	1.6 b	242.3 b	69.5 a	4.3 a
HYD		197.6 a	0.1 b	0.3 b	118.6 a	333.3 a	145.5 a	39.6 a	676.6 a	11.1 b	0.0 b
Crop											
Cherry Tomato		134.2 b	3.9	1.6	76.5	181.6	75.6	20.0	376.6	35.2	0.0 b
Basil		150.9 ab	4.3	1.6	70.2	204.5	81.5	20.4	403.4	45.3	0.9 b
Lettuce		176.0 a	4.2	1.6	76.3	226.6	83.2	20.3	399.8	40.4	5.6 a

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean of 6 replicates.

ns, *, **, *** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 8. Mineral nutrient concentrations in different plant tissues of tomato, basil, and lettuce grown in either an aquaponic or hydroponic system.

Analysis of variance	Total N	Total P	K	Ca	Mg	SO$_4$–S	Na	Cl
				(mg g^{-1})				
System								
AQU	31.1 b	18.1 b	59.5	4.9 b	3.8 b	6.9 b	8.2 a	5.7 a
HYD	36.4 a	20.8 a	54.5	6.2 a	5.5 a	23.8 a	2.5 b	2.4 b
Crop								
Cherry Tomato	29.3 b	16.9 c	57.4 ab	5.6 a	7.0 a	9.9 b	5.3 ab	3.9 ab
Basil	32.2 b	19.8 b	49.0 b	6.9 a	3.1 b	33.7 a	3.8 b	2.9 b
Lettuce	44.0 a	24.6 a	68.0 a	3.4 b	2.3 b	0.0 b	7.7 a	6.1 a
Tissue								
Leaves	40.7 a	20.2 b	72.3 a	7.0 a	7.3 a	7.3 b	6.0 ab	5.9 a
Stems	20.2 b	14.8 c	65.4 a	4.6 b	5.5 ab	34.3 a	3.3 bc	3.3 b
Roots	37.5 a	24.4 a	36.3 b	5.2 ab	3.0 bc	16.2 ab	7.3 a	2.7 b
Fruits	25.5 b	15.1 c	56.5 a	3.9 b	0.0 c	0.08 b	1.8 c	3.9 ab
System	**	***	ns	*	*	**	***	**
Crop	***	***	**	***	***	***	**	***
Tissue	***	***	***	ns	***	*	***	***
System × Crop	ns	ns	ns	***	***	ns	***	***
System × Tissue	***	ns	***	ns	***	ns	***	***
Crop × Tissue	***	***	***	***	***	***	***	***
System × Crop × Tissue	***	***	***	***	***	***	***	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05). Each value in the table is the mean of 6 replicates.

ns, *, **, *** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
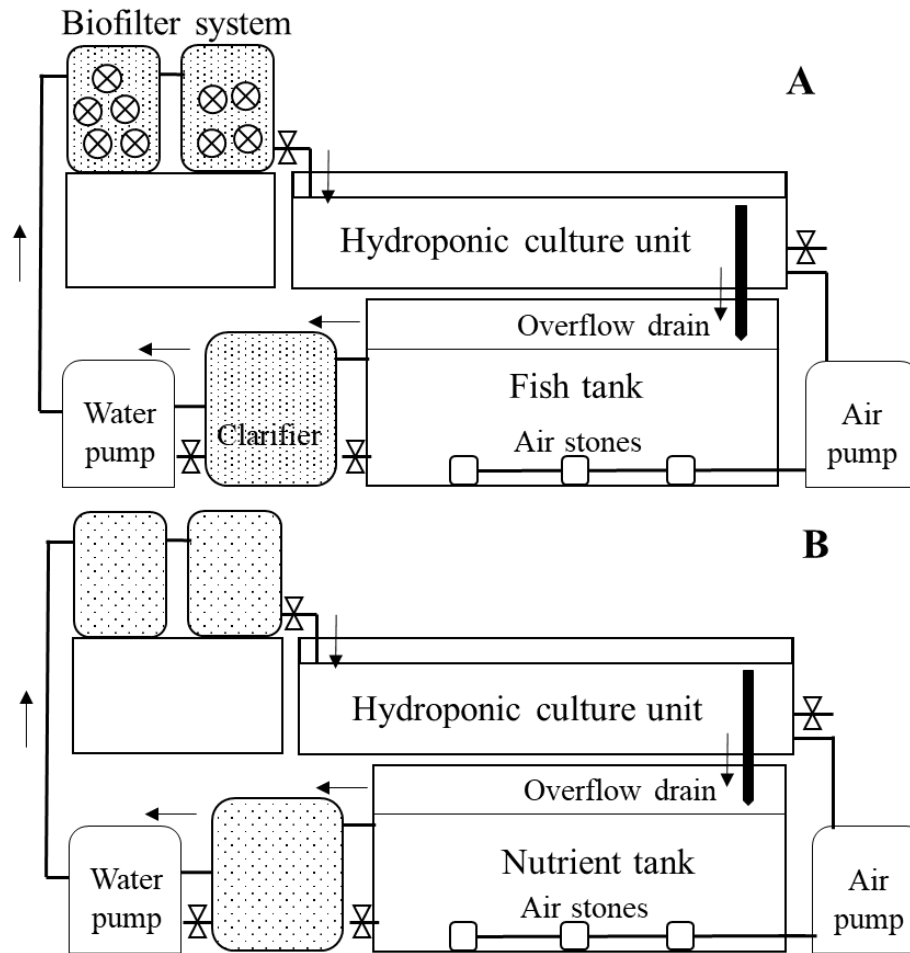
Figure 1. Schematic diagram of experimental units: (A) aquaponic system; (B) hydroponic system.
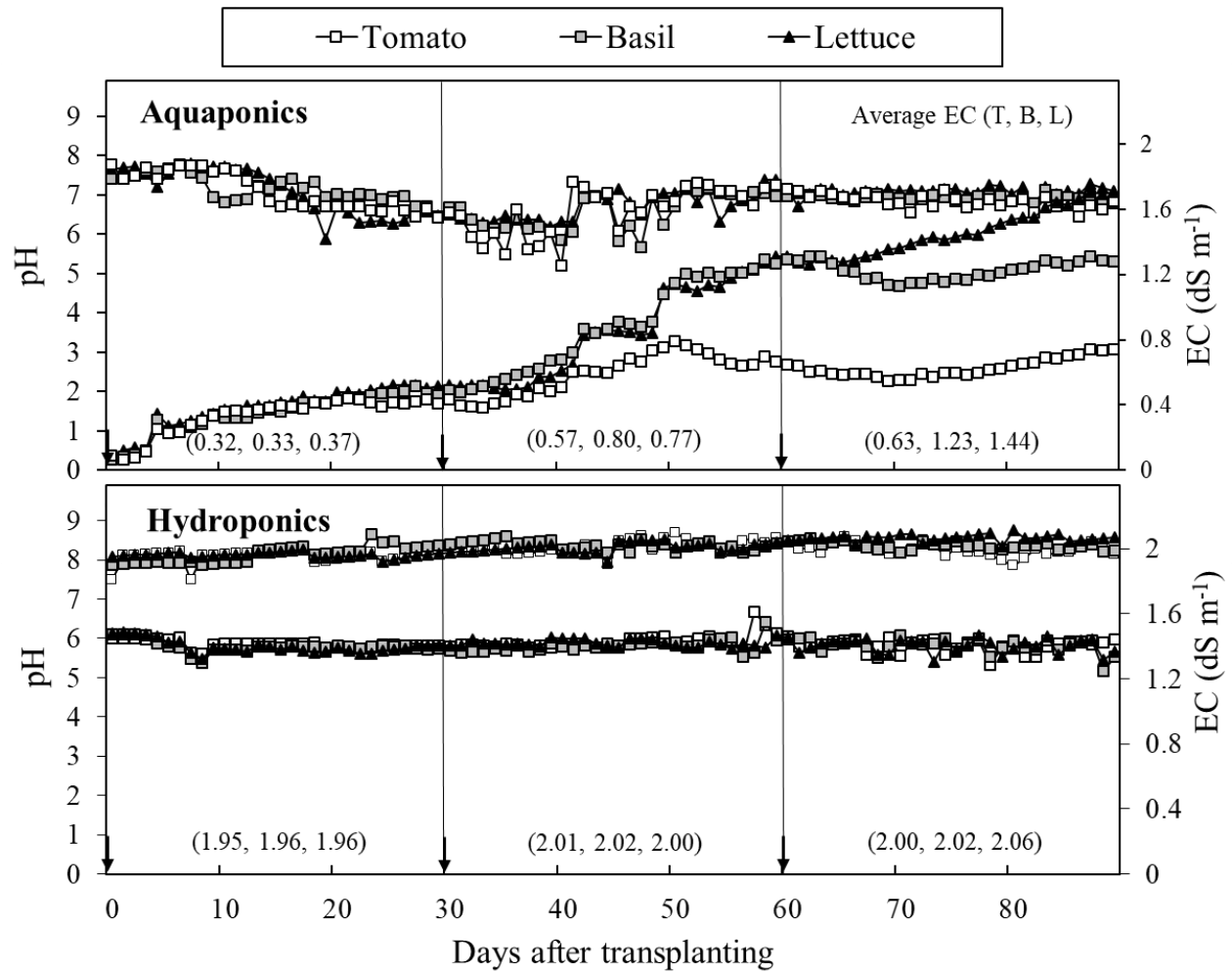
Figure 2. Variations in electrical conductivity (EC; dS m^{-1}) and pH in tomato-, basil-, and lettuce-based aquaponics and hydroponics over a three-month production period. EC of aquaponics was monitored and the pH was adjusted to 7 daily, while EC and pH of hydroponics were adjusted daily to 2 and 6, respectively. Nutrient solutions were recirculated throughout 3-month experimental period. Arrows above the different time points indicate where lettuce seedlings were transplanted into the system (once every month). Tomato and basil seedlings were transplanted once at Day 0. Average EC levels of tomato (T)-, basil (B)-, and lettuce (L)-based systems were given for each time period (one month). EC and pH were measured and averaged at two different locations (aquaponics: fish tank and hydroponic culture unit; hydroponics: nutrient reservoir and hydroponic culture unit) in the system. Each data point is the mean of 3 replicates.
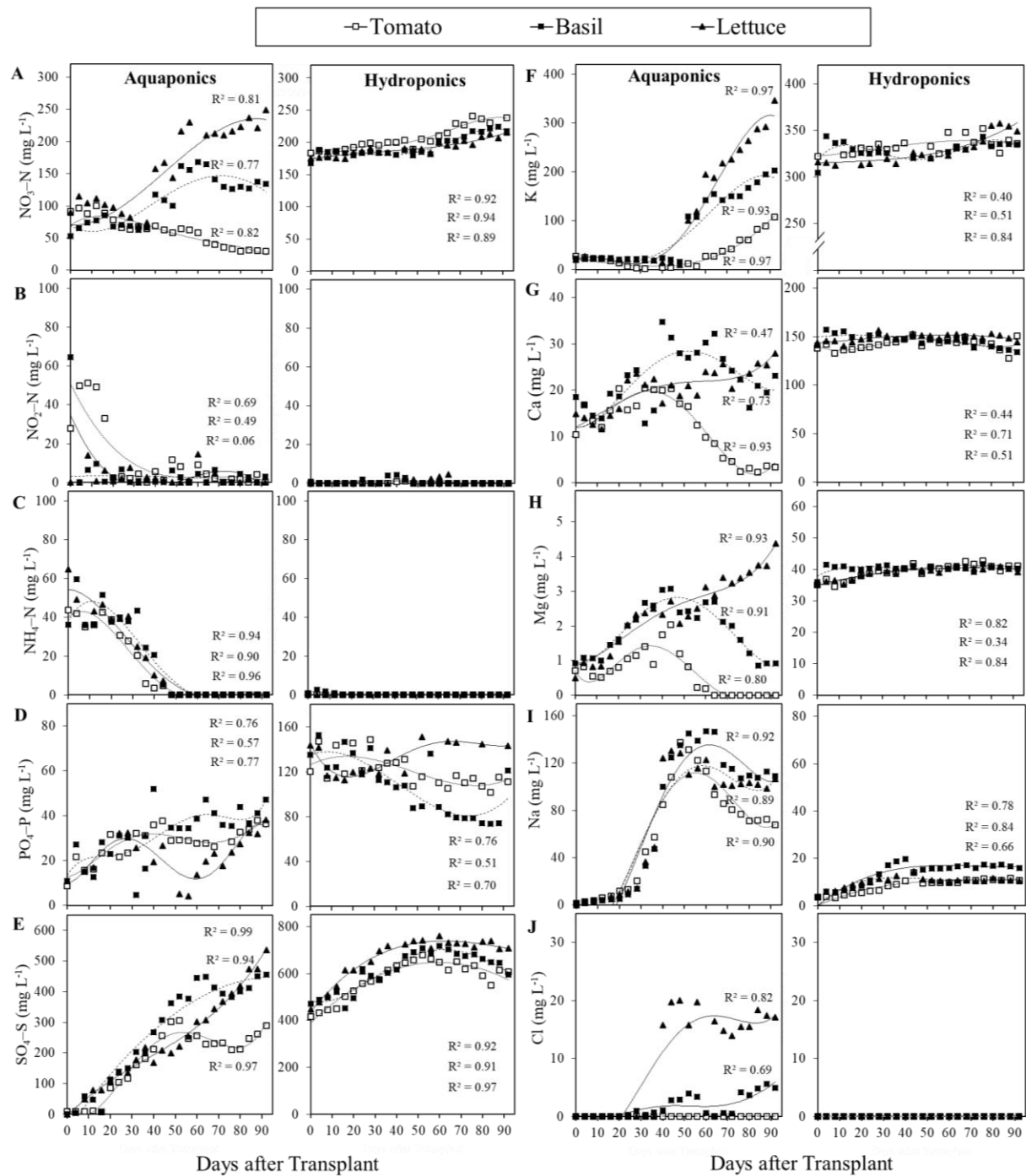
Figure 3. Changes in macronutrient (A–J) levels in the aquatic phase of tomato-, basil-, and lettuce-based aquaponic and hydroponic systems during a 3-month production period. Water samples were collected once every two days at two different locations in the system (aquaponics: fish tank and hydroponic culture unit; hydroponics: nutrient reservoir and hydroponic culture unit), analyzed using an ion chromatography, and averaged per replicate. Each data point is the mean of 3 replicates.
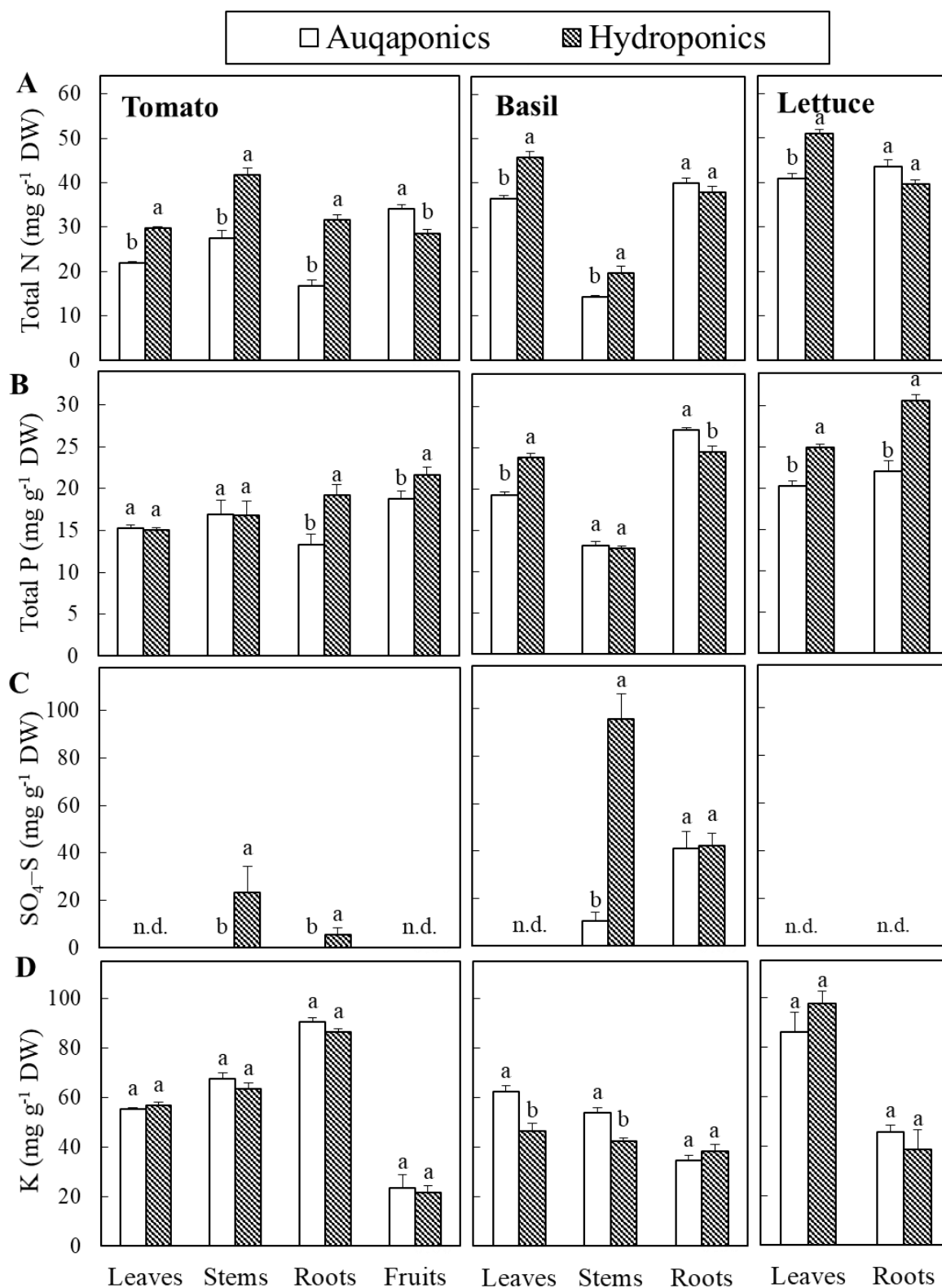
Figure 4. Total nitrogen (A), total phosphorus (B), sulfate (C), potassium (D) concentrations in the leaves, stems, roots, and/or fruits of tomato, basil, lettuce grown for 3-, 3-, and 1-month(s) in an aquaponic or hydroponic system. Data represent mean values ± SE (n = 6).
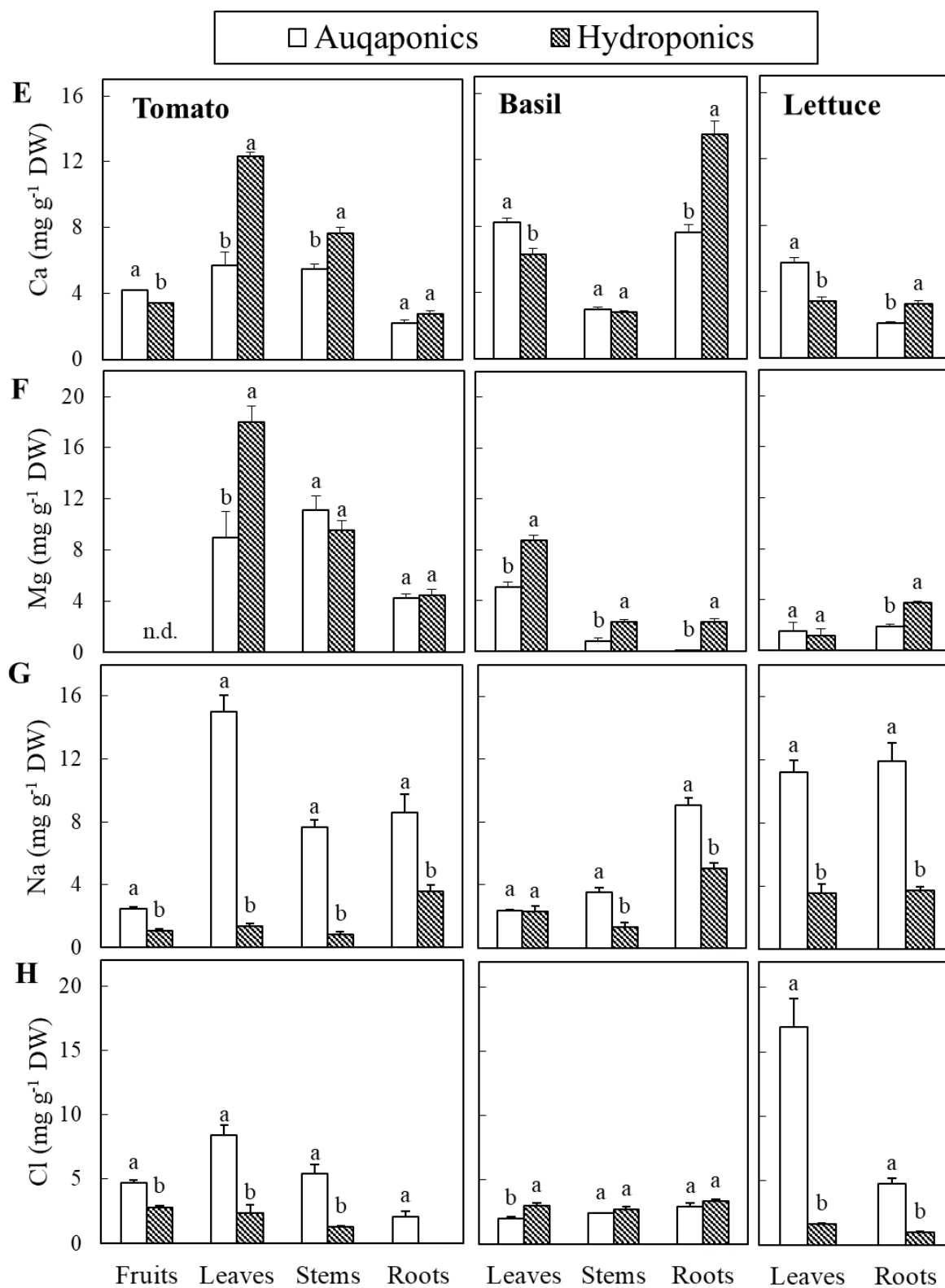
Figure 5. Calcium (E), magnesium (F), sodium (G), and chloride (H) concentrations in the leaves, stems, roots, and/or fruits of tomato, basil, and lettuce grown for 3-, 3-, and 1-month(s) in an aquaponic or hydroponic system. Data represent mean values ± SE (n = 6).

Table S1. Macro- and micro-nutrient compositions and concentrations used in hydroponics and aquaponics.

Parameter	Hydroponics[a]		Aquaponics[b]
	Basil/Lettuce	Tomato	
Macronutrient (%)			
Total nitrogen (N)	0.043	0.044	> 6.88
P_2O_5–P	0.093	0.130	> 1.10
K_2O–K	0.035	0.034	0.99
SO_4–S	–	–	0.43
Ca	0.075	0.075	2.25–2.75
Mg	0.039	0.037	0.23
Micronutrient (mg kg^{-1})			
B	2.00	2.75	–
Cu	1.05	0.95	10
Fe	21.00	10.00	40
Mn	1.90	8.00	80
Mo	0.42	0.40	–
Zn	2.10	2.70	153

All the information come from related company.

"–" means "not contain" or no related information.

[a] Nutrient compositions of fertilizer used in hydroponics were calculated based on 1:100 dilution of commercial fertilizer.

[b] Nutrient compositions of fish feed used in aquaponics were calculated based on g feed per day.

Table S2. Pearson's correlation coefficients of nutrient (total N, Ca, and Mg) concentrations in the leaves of tomato, basil, and lettuce for the SPAD value.

Treatment		Regression coefficient			
Crop	Variables	Total N	Ca	Mg	SPAD
Tomato	Total N	—			
	Ca	0.93***	—		
	Mg	0.86***	0.93***	—	
	SPAD	0.82***	0.78**	0.65*	—
Basil	Total N	—			
	Ca	–0.19ns	—		
	Mg	0.55ns	–0.04ns	—	
	SPAD	0.80**	–0.54ns	0.48ns[a]	—
Lettuce	Total N	—			
	Ca	–0.22ns	—		
	Mg	–0.22ns	0.84***	—	
	SPAD	0.59*	–0.51ns	–0.31ns	—

ns, *, **, *** mean no significant or significant at P<0.05, 0.01, or 0.001, respectively.

[a] Significant at $P < 0.12$
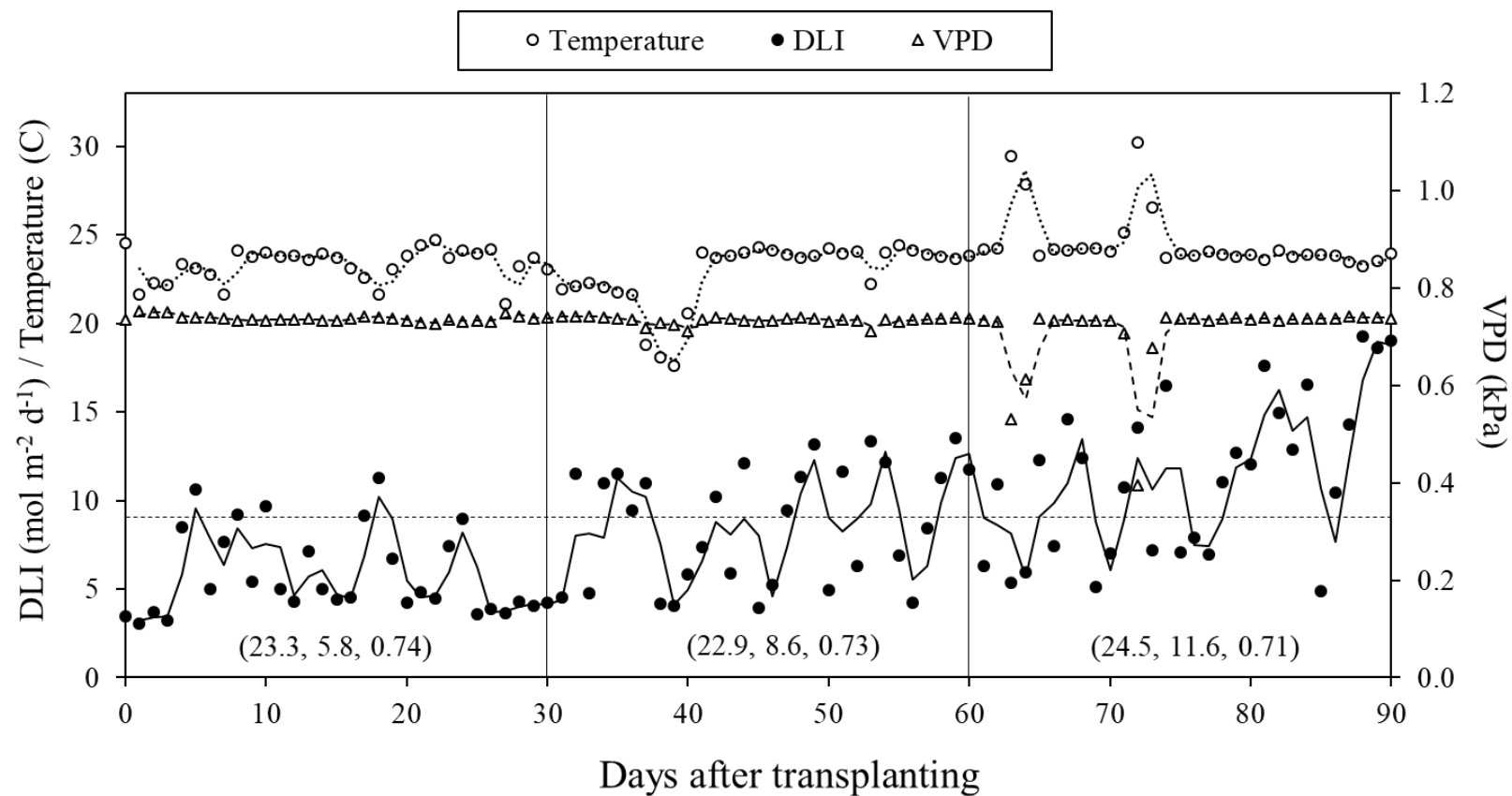
Figure S1. Ambient temperature, daily light integral (DLI), and vapor pressure deficit (VPD) collected in the greenhouse during the experimental period (December through February). The parameters were averaged over the day. A dotted line is the average DLI during entire production period (8.9 mol m^{-2} d^{-1}).

NUTRIENT-MANAGEMENT REGIME AFFECTS WATER QUALITY, CROP GROWTH, AND NITROGEN USE EFFICIENCY OF AQUAPONIC SYSTEMS

Abstract

Sustainable nutrient management is of critical importance to achieve high crop yield and quality and to improve nutrient use efficiency in agricultural production systems but has not been fully established for aquaponics. The objective of this study was to determine the effects of fish feeding regime on water quality, crop performance and yield, and nitrogen (N) use efficiency in recirculating aquaponic systems. The same amount of total N (120 g) was applied to aquaponics with different feeding regimes: aquaponic increasing feeding (AIF; the standard feeding regime), uniform feeding (AUF), and intermediate feeding (AMF), for one-month production of six vegetable and herb species. Crops grown in AIF and AUF showed contrasting results in yield and SPAD value (chlorophyll content), and therefore were further evaluated for nutrient profile in aquaponic solution and crop growth and performance compared to those in hydroponics (HYD), using eight leafy vegetable (Chinese cabbage, Mizuna, Swiss chard, lettuce, pac choi), herb (basil, chia), and fruity vegetable (cherry tomato) species. AUF improved water quality by reducing average concentrations of harmful compounds (i.e., NO_2–N and Na) compared to AIF and crop growth and yield similar to those of HYD. Particularly, AUF tended to increase concentrations of mineral nutrients (i.e., NO_3–N, PO_4–P, Ca, and Mg) in aquaponic solution during the first week after transplanting, while decreasing the concentrations of harmful compounds in comparison to AIF. Regardless of feeding regime, aquaponics reduced fine root growth in leafy vegetables and herbs, compared to hydroponics. Overall, vegetables and herbs grown in AUF had a greater photosynthetic rate (*Pn*) from the first to the second week after transplanting and throughout production period and showed higher SPAD value and leaf total N content to the level similar to or slightly lower than HYD. Consequently, AUF increased N use efficiency (NUE) of the system by 30% and up to 600% compared to those in AIF and HYD, respectively. In conclusion, aquaponic crop production and N use efficiency can be increased by uniform feeding regime as it improves water quality and nutrient availability for better seedling establishment, consequently enhancing quality and/or yield of vegetables and herbs in aquaponics.

1. Introduction

Aquaponics is a rapidly emerging agricultural production system, which recycles effluent from aquaculture to produce plant crops with spent nutrients by creating a symbiotic ecosystem for fish, microbes, and plants in a closed system (Martins et al., 2010). Aquaponics has been proposed as a sustainable solution to the current challenges in food production, as it recycles more than 98% of their water from aquaculture effluents (Al-Hafedh et al., 2008), and therefore dramatically reduce the amount of wastewater discharged to the environment. Although recirculating aquaponic systems are known to be more efficient in the utilization of water and nutrients than conventional systems (Barbosa et al., 2015), management of a recirculating aquaponics is a challenge due to

water quality management issues (Badiola et al., 2012), which need to be properly addressed to improve nutrient recycling and crop yield in the system. For example, while each organism requires different water environment for optimum growth, it is suggested that reconciling water quality parameters is a necessary compromise to promote nitrification to ensure fish health, and therefore a pH within the range of 6.5 to 7.5 is usually maintained in aquaponic systems (Tyson et al., 2007). Such water environment is suboptimal for plant growth, making plant crops less productive and profitable compared to those grown in hydroponics (Chapter 2, this dissertation; Quagrainie et al., 2018). It is important to produce high quality and high yielding crops in aquaponics as healthy, actively growing plants can act as a biological filter and improve water quality by removing nutrients from aquaponic solution, and this, in turn, contributes to maintaining fish health and wellbeing (Yildiz et al., 2017). Despite the high production potential and the importance of sustainable food production, lower yield and quality of plant crops have been often reported in aquaponics. Leaf chlorosis or yellowing was observed in lettuce (*Lactuca sativa* 'Cherokee'), basil (*Ocimum basilicum* 'Genovese'), cherry tomato (*Lycopersicon esculentum* 'Washington 83 Cherry') (Chapter 2, this dissertation), tomato (Roosta and Hamidpour, 2011), and eggplant (*Solanum melongena* L.) (Roosta and Mohsenian, 2015). Leaf yellowing in aquaponics is often considered attributable to high pH of aquaponic solution which is typically higher than the optimal range of 5.5 to 6.5 in hydroponics (Hochmuth, 2013; Resh, 2013) and/or limited availability of mineral nutrients for plants when fish waste is used as the sole source of nutrients, which include phosphorus (P), potassium (K), iron (Fe), manganese (Mn), and sulfur (S) (Seawright et al., 1998; Rakocy et al., 2004). Supplementation with potassium (K), sulfur (S), iron (Fe), and manganese (Mn) to aquaponic solution or application as a foliar spray has been suggested to increase crop growth and yield (Rakocy et al., 2004; Rakocy et al., 2006; Roosta and Hamidpour, 2011). However, the suboptimal concentration of calcium (Ca), magnesium (Mg), and/or nitrate (NO_3–N) from ingested fish feed was considered as one of the major contributors to leaf yellowing and low yield as plants require these macronutrients in large quantity (Chapter 2, this dissertation). Lower levels of these mineral elements can occur especially when high-quality water is used in aquaponics or the system is relatively young, lowering crop yield and quality in aquaponics (Chapter 2, this dissertation; Pantanella et al., 2012).

Despite yield and quality compromise under the current management regime, there are no recommendations for nutrient management practices available for recirculating aquaponic systems. Plants require at least 14 mineral elements for adequate nutrition (Marschner, 2012): the macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) and the micronutrients such as chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni), and molybdenum (Mo). Crop production is not only limited by low availability of these essential mineral elements but also by the presence of excessive concentrations of potentially toxic elements such as sodium (Na), Cl, B, Fe, Mn and aluminum (Al) in the rhizosphere (White and Brown, 2010). Significantly higher levels of NO_2–N and Na were demonstrated in aquaponics compared to hydroponics, which may be a major concern for limiting crop growth and yield in aquaponic systems (Chapter 2, this dissertation). Short-term exposure to a deficient level in any of the mineral nutrient elements or an excess level in harmful elements during plant growth and development may negatively impact plant yield and quality in aquaponics.

Nutrient availability and composition in aquaponics are mainly affected by the feeding regime as fish feed is the major source of nutrients for plant crops. The current recommended feeding guideline for aquaponics is to provide fish feed at 1 percent of body weight per day for fish of more than 100 g of body mass (Somerville et al., 2014). The feeding rate ratio is 40–50 g/m^2 for leafy greens; and 50–80 g/m^2 for fruiting vegetables and gradually increased by 1 to 2% of their body weight per day (Somerville et al., 2014). As such, increasing feeding rate has been used as a standard management regime for aquaponic commercial operations and research. This concept may have been derived from the assumption that the application of additional nutrients is beneficial to boost plant growth and yield, as aquaculture wastewater does not contain sufficient level of nutrients for plants. Although being widely used, it is not known if the standard increasing feeding regime is the best nutrient management practices in aquaponics.

It is well recognized that plant displays a sigmoid pattern of determinate growth (Yin et al., 2003). For example, lettuce displays about 7 to 10 days latent period of growth, followed by rapid leaf expansion and biomass gain period during which plants require higher levels of nutrients. In commercial hydroponic production, the crop is harvested immediately after growth reaches the maximum. However, such growth pattern and nutrient demand may not be well aligned with nutrient availability in aquaponic solution as the standard feeding practices are designed based on fish weight gain. Fish feed is one of the top costs for aquaponic operation (Quagrainie et al., 2018) and ingested fish feed is the major source of fertilizer for plant crops in aquaponics. There is a critical need to utilize nutrient source more efficiently and manage aquaponic systems more effectively to cultivate crops in order to improve the nutrient use efficiency of the system. Proper nutrient management practices will enhance high yield and quality of plant crops in an aquaponic system and improve water quality for fish by minimizing harmful compounds in aquatic solution, which is important for the economic sustainability of recirculating aquaponic systems.

The objectives of this study were: (1) to evaluate three different feeding regimes, the standard feeding regime, aquaponic increasing feeding (AIF), and two alternative feeding regimes, uniform feeding (AUF) and intermediate feeding (AMF) for vegetable and herb production in recirculating aquaponic systems, and (2) to determine the effects of feeding management regime on water quality, crop performance, quality, and yield, and N use efficiency in recirculating aquaponics in comparison to hydroponics.

2. Materials and methods

2.1. System design

Two experiments were conducted in the greenhouse at Purdue University at West Lafayette (40º 148 25' 26.4'' N, 86° 55' 44.4'' W). Each aquaponic system was equipped with a 350 L fish tank, a 20 L clarifier, two 20 L biofilter tanks and a 350 L (1.0 m^2) hydroponic culture unit (**Figure 6 A**).

Each hydroponic system was equipped with a 350 L nutrient solution reservoir, a 20 L clarifier, two 20 L biofilter tanks and a 350 L (1.0 m^2) hydroponic culture unit (**Figure 6 B**). Peristaltic

pumps (Masterflex, Cole-Parmer, USA) were used to recirculate the aquaculture effluent or nutrient solution within the system unit. Total water volume in each aquaponic and hydroponic unit was 700 L and the flow rate was set at 138 L/h, giving a water retention time of 300 min in fish tank/nutrient solution reservoir and hydroponic culture unit. Aquaculture effluent or nutrient solution flowed into biofilter after passing through the clarifier, which was designed to capture the majority of suspended solids, and then hydroponic culture unit. Deep-water culture system was used in the hydroponic unit. Plants were held up by a foam board set on the top of the edges of the hydroponic unit. Each hydroponic unit and fish tank/nutrient solution reservoir had air stones to maintain dissolved oxygen (DO) concentrations above 5 mg/L. The fish tanks and nutrient solution reservoirs were covered with a plastic board to prevent algal growth. There was also a lid on each board which could be open to permit light to the tank during the daytime. Nutrients dissolved in the aquaculture effluent and nutrient solution were absorbed by plants in the hydroponic unit and purified water was then recirculated into the fish tank and nutrient solution reservoir, respectively. Water was recirculated between the fish tank or nutrient solution reservoir and hydroponic culture unit and was not exchanged during the study period except for replenishing evapotranspiration losses by reverse osmosis (RO) water.

The photoperiod was 14-h (8:00 am to 10:00 pm) consisting of natural daylight with supplemental lighting using high-pressure sodium (HPS) lamps (600-W, P.L. Light Systems Inc., Beamsville, ON, Canada). A supplemental photosynthetic photon flux (PPF) of the greenhouse was measured using a quantum sensor (LI-250A light meter; LI-COR Biosciences, Lincoln, NE) and photosynthetically active radiation in the greenhouse was averaged at 134 μmol m^{-2} s^{-1}. Day (8:00 am to 10:00 pm) and night (10:00 pm to 8:00 am) temperatures were set at 24 and 18°C, respectively, with an hour transition between two temperature regimes. Depending on ambient temperature, the greenhouse was cooled as needed using a fan-and-pad evaporative-cooling system, heated using radiant hot-water-pipe heating, and retractable shade curtains regulated by an environmental control system (Maximizer Precision 10, Priva Computers Inc., Vineland Station, ON, Canada). Environmental data for greenhouse ambient daily light integral (DLI), ambient temperature, and vapor pressure deficit (VPD) were averaged per day and presented in **Figure S2**.

2.2. Plant and fish materials

In each aquaponic system, the fish tank was stocked with Nile tilapia (*Oreochromis niloticus*) fish obtained from Animal Sciences Research and Education Center at Purdue University where the fish had been grown for 4 months in a conventional aquaculture system. Stocking density was shown in **Table 9** and slightly varied between two experiments because of fish weight gain. The fish feed used in this study was a complete diet containing 41% protein and 1.1% phosphorus (AquaMax Sport Fish 500, Purina Mills, St. Louis, MO) with 4.8-mm floating pellets (**Table S3**).

Six and eight varieties of vegetables and herbs were examined in experiment 1 and experiment 2, respectively: leafy vegetable (Chinese cabbage, lettuce, mizuna, pak choi, Swiss chard); herbs (basil, chia), and fruity vegetable (cherry tomato) (**Table 10**). Seeds were purchased from a commercial source (Johnny's Selected Seeds, Winslow, ME) and sown in agrifoam (SteadyGROWpro, Syndicate Sales, Inc., Kokomo, IN) trays with few days interval in order to

match the size of seedlings at the time of transplanting. Seeds were germinated in a climate room under 150 µmol m^{-2} s^{-1} using full-spectrum LEDs (320-W "VYPRx", Fluence Bioengineering, Inc., Austin, TX) for 18 h d^{-1}. Seeds were irrigated initially with tap water, followed by a half-strength fertilizer solution once germinated, and full-strength fertilizer after seedlings developed true leaves (Kim et al., 2018). The seedlings were transferred to a glass-glazed greenhouse and grown under supplemental lighting using overhead high-pressure sodium (HPS) lamps (600-W, P.L. Light Systems Inc., Beamsville, ON, Canada) for 14-h photoperiod (8:00 am to 10:00 pm).

The fertilizer was applied with irrigation as necessary with a combination of two water-soluble fertilizers (3:1 mixture of 15N–2.2P–12.5K Cal-Mag Special and 21N–2.2P–16.6K Multi-Purpose fertilizers, respectively; Everris NA, Dublin, OH). The fertilizer consisted of (mg/L): 150 nitrogen (N), 20 phosphorous (P), 122 potassium (K), 38 calcium (Ca), 15 magnesium (Mg), 0.8 iron (Fe), 0.4 manganese (Mn) and zinc (Zn), 0.2 copper (Cu) and boron (B), and 0.1 molybdenum (Mo). Nitrate form was 76% of nitrogen provided. After the third true leaf of seedlings emerged, uniform healthy seedlings were randomly selected for each treatment and transplanted into mesh pots each containing 85g clay pebbles, then transferred to a hydroponic unit of aquaponic or hydroponic systems.

2.3. Nutrient management regimes

Feeding rates were determined based on the initial weight of fish and water quality parameters (i.e., total ammonia nitrogen (TAN), NO_2-N) in aquaponic solution as described in 2.4, and therefore, actual feeding rates were lower than what was normally suggested for aquaponics, which was necessary to maintain water quality and fish health (**Table 9**). For example, in experiment 1, the initial fish weight was within the range of 100 and 200g; however, the initial and final feeding rate in our study was 0.5 and 0.8%, 0.7 and 0.7%, and 0.8 and 0.7% for AIF, AMF, and AUF, respectively. Since the initial fish weight in experiment 2 was nearly 240g, and the actual feeding rates were 0.4 and 0.6% for AIF and AUF, respectively (**Table 9**). The reduced feeding rate with a greater fish weight reflects suggested aquaculture feeding guidelines (New, 1987). Feed conversion ratio (FCR) was calculated by the following formula: FCR = Total feeding amount (g) / Fish biomass increment (g).

Experiment 1: Fish were fed once per day at 9:00 am daily at the designated feeding rate of three different feeding regimes: increasing feeding (AIF), moderate feeding (AMF), and uniform feeding (AUF). Total feeding amount was the same for three feeding regime treatments, and the difference among the treatments was the initial and final feeding rates as outlined in **Table 9**. AIF is the standard feeding regime in aquaponics and was the control in this experiment. AMF is a moderate increasing feeding with less difference between initial and final feeding rates compared to AIF. Fish weight was measured at the beginning, weekly, and at the end of the experiment by carefully removing an individual fish from the fish tank and transferring them into a bucket filled with water. This procedure was repeated until the weight of at least 50% fish in a fish tank was measured. The average fish weight was used to determine the initial amount of feed and to make adjustments on the feed amount weekly. AUF had a higher initial feeding rate compared to other feeding regimes, with a uniform amount of feed throughout one-month crop production. During

4-week plant crop production, the total amount of fish feed was the same among the treatments (1800g), which accounted for 120g total N.

Experiment 2: Two contrasting aquaponic feeding regimes, increasing feeding (AIF) and uniform feeding (AUF), were chosen based on the results from experiment 1 and compared with hydroponic treatment (HYD). In each hydroponic system, nutrient solution reservoir and hydroponic culture unit were filled with commercial nutrition solutions (**Table S3**) which were used as an initial and replenished nutrition solution. During 4-week plant crop production, the total amount of fish feed was the same among the feeding treatments (1800g) accounting for 120g total N. In contrast, total N used in HYD accounted for 720g, which was nearly 5 times higher than that in aquaponics (**Table 9**).

2.4. Measurement of water quality parameters

Water quality parameters, such as temperature (T; °C), pH, electricity conductivity (EC; mS/cm), and dissolved oxygen (DO; mg L^{-1}) of the fish tank and hydroponic culture unit were measured daily before feeding at 9:00 am using HQ40d portable water quality lab package (HACH Corp., Loveland, CO, USA). Aquarium heaters (Eheim Jager TruTemp, Germany) were used to maintain the water temperature of the fish tanks within the optimum range (26–28°C) for tilapia culture in aquaponic systems. In hydroponics, EC was maintained at around 2 mS/cm by adding and replenishing nutrient solution daily. A mixture of potassium hydroxide (1N) and saturated (0.05N) calcium hydroxide (v:v=1:1) was directly added to the fish-tank to adjust pH at around 7 daily before feeding, while pH in hydroponics was adjusted to 5.5 to 6.0 by directly adding the solution mixture to the nutrient solution reservoir.

Water samples were collected from the fish tank and hydroponic culture unit every 3 days before feeding, and were analyzed immediately for TAN, NO_2–N, NO_3–N, and PO_4–P concentrations, using HACH reaction kits (Loveland, Co. Ltd., USA), namely Ammonia Reagent Powder Pillows, Nitrite Reagent Powder Pillows, Nitrate Reagent Powder Pillows and Phosphate Reagent Powder Pillows, respectively. The same water samples were used to analyze macronutrients (i.e., NO_3–N, NO_2–N, NH_4–N, PO_4–P, K, Ca, Mg, SO_4–S, Na, Cl) using ion chromatography (Dionex ICS–5000, Thermo scientific, Co. Ltd., USA) as described in 2.8. Dynamic changes in macronutrient levels was worked out by using quadratic regression analysis (**Figure 8**).

2.5. Growth measurements

Crop growth parameters were measured weekly, which included plant height, leaf number, leaf length, leaf area, and SPAD (soil plant analysis development) value (an index of chlorophyll content per unit leaf area). The SPAD readings were taken weekly on each young fully expanded leaf using a SPAD-502 Chlorophyll Meter (Minolta Camera Co. Ltd., Japan). Five readings per leaf were taken at the central point of a leaf between the midrib and the leaf margin for leafy vegetable and herb, and the terminal leaflet for tomato and the values were averaged. Leaf temperature was measured at the third week after transplanting using a hand-held infrared radiometer (MI-210, Apogee Instruments, Inc., Logan, UT, USA) at a distance of approximately 4.8 cm from the leaf surface.

At harvest, all plant samples were divided into different plant tissues (roots, stems, leaves, and/or flowers and fruits), and weighed for fresh weight. All leaf samples were scanned for leaf area by using a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA) immediately after harvest. Plant samples were oven-dried (over 72 h at 70 °C) and weighed for dry weight. All dried sample were filtered through a 10-mesh sieve after grinding with a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ, USA) and kept in plastic vials for nutrient analysis. At the beginning and the end of the study, fish were randomly selected to measure fresh weight as described in 3.2.

2.6. Measurements of root morphological traits

Plant root samples were stored in 50% ethanol solution for root length, diameter, surface area, volume measurement using WinRHIZO root-scanning system (WinRhizo Pro v.2005b, Regent Instruments, Québec, Canada). The debris removal filter was set to discount objects less than 1 cm^2 with a length/width ratio less than 4. The scanned images were then used to determine root morphological traits using WinRHIZO Pro software (Regent Instrument Inc., Quebec City, Quebec, Canada). Diameter class length (root length within a diameter class) were generated in the images of adventitious roots acquired from the system. The roots were divided into 26 diameter classes at 0.25 mm intervals and root length per each root diameter class was calculated. The root diameter class distribution was computed based on the proportion of the root length in each root diameter class compared to the total root length.

2.7. Measurement of photosynthetic properties

Gas-exchange measurements were performed using a portable gas exchange system (LI-6400XT; LI-COR Biosciences, Lincoln, NE) equipped with a 6-cm^2 leaf chamber with built-in LEDs (470 and 665-nm peak wavelengths for blue and red LEDs, respectively) on recently fully expanded leaves at each canopy level. Illumination was supplied at a PPF of 400 μmol/m^2/s by red and blue LEDs at a ratio of 9:1 under ambient temperature conditions when supplemental lighting was in use. The reference CO_2 concentration and flow rate through the chamber were 400 μmol/mol and 500 μmol s^{-1}, respectively.

One leaf at each canopy level was selected from each plant for the measurements. The measurements of photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and internal CO_2 (Ci) were conducted between 9:00 am and 14:00 pm at a PPF of 400 μmol/m^2/s. Readings were taken when the coefficient of variation (i.e., sample CO_2, sample H_2O, and flow rate) was less than or equal to 0.2%, which typically occurred within 10 min. The intrinsic water use efficiency (WUE) was calculated by dividing Pn by gs (Chaves et al., 2004).

2.8. Anion and cation measurements

For anion and cation nutrient analysis of water samples, frozen water samples kept in a –20°C freezer were thawed under room temperature and centrifuged immediately at 12000 rpm for 10 min, and then liquid supernatants were collected and subjected for cation and anion nutrients measurement. For cation nutrient analysis of dried plant samples (Basta and Tabatabai, 1985), each sample was weighed to the nearest 0.100 g and placed in a 20 mL glass vial with three drops of 5%

H$_2$SO$_4$ in ethanol, then ashed in a muffle furnace at 550 °C for 3 hours. After the process, 8 mL aliquot of 5 mM HCl was added, vortexed for 10 seconds, heated near boiling (90°C), then vortexed again. Plant samples were centrifuged at 12000 rpm for 10 minutes, and then liquid supernatants were collected. For anion nutrient analysis of dried ground plant samples (Beke and Selles, 1993), each sample was weighed to the nearest 0.1 g and placed in a 50-mL centrifuge tube with 0.1 g decolorizing carbon and 13.3 mL Millipore water. Then samples were vortexed for 10 seconds and shaken for 30 minutes. The samples were centrifuged at 12000 rpm for 10 min, and liquid supernatants were collected. After being diluted to a desirable range, each sample was prepared into an autosampler vial for injection.

The nutrient compositions and concentrations of processed water samples were analyzed by the ion chromatography system (Thermo Scientific Dionex ICS–5000, Waltham, US) equipped with capillary pumps, electrolytic eluent generation modules, injection valves, capillary electrochemical suppressors, cation column (IonPac CS12A) and anion column (IonPac AS18 column), and conductivity detectors to determine the concentration of cations (including ammonium, magnesium, calcium, potassium, sodium) and anions (including nitrite, nitrate, phosphate, sulfate, chloride). The column temperature was maintained at 20˚C.

The IC was coupled to an AS–AP autosampler (Thermo Scientific, Waltham, US), allowing for continuous sample loading and injection in sequence including standards and samples. Conditions for IC anion analyses were as follow: eluent (23 mM KOH) flow rate was set at 1 mL/min and suppressor current was set at 57 mA and raised to 99 mA during gradient runs of the eluent (40 mM KOH), which was conducted for optimal phosphate analysis. A gradient elution method was employed in which eluent concentration was increased from 23 to 40 mM at 12 minutes, remained at 40 mM for 3 minutes, and then decreased to 23 mM for 4 minutes. Conditions for IC cation analyses are as follows: eluent (20 mM MSA) flow rate was set at 1 mL/min for isocratic runs and suppressor current was set at 59 mA. Chromeleon data management software (version 7.1) was used for data processing.

2.9. Total nitrogen measurement

For total nitrogen analysis of each plant sample, 30 mg ground sample was measured and transferred into an empty sample tin using a clean small sampling spatula, then the tin was carefully wrapped up into a ball. The total nitrogen content of a sample was then measured by using the C/N analyzer (FlashEA 1112, Thermo Fisher Scientific, Waltham, MA, USA). Nitrogen use efficiency (NUE) was calculated by the following formula: NUE= $(N_f − N_u/N_a) \times 100\%$, N_f= the total nitrogen accumulation in the final harvest plant tissue (g); N_u= the total nitrogen accumulation in the initial plant tissue (g); and N_a= the quantity of total nitrogen applied (g).

2.10. Experimental design and data analysis

The experimental design was a randomized complete block design with the management regime and plant species as the main factors. The study was conducted from November 2016 to April 2017. Each trial was conducted for one month. In experiment 1, six independent aquaponic units were operated in each trial. The experiment was repeated twice and each trial consisted of three

treatments (AIF, AMF, and AUF) with two system replicates. There were six plant species and each plant species had three sample replicates in each aquaponic system. In experiment 2, four aquaponic units and two hydroponic units were operated in each trial. The experiment was repeated three times and each trial consisted of three treatments (AIF, AUF, and HYD) with two system replicates. There were eight plant species and two sample replicates in each system. Results from each trial showed similar trends in both experiments, and therefore, the data sets were pooled for further analyses. All data were statistically analyzed using JMP® for Windows, Version 13.2 (SAS Institute Inc., Cary, NC). Statistical differences were determined using a two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test or Student's t-test at P ⩽ 0.05.

3. Results

3.1. Water quality parameters

Average water quality parameters of aquaponics in experiment 1 and 2 were 7.7 and 7.1 mg L^{-1} dissolved oxygen (DO), pH 6.7 and 7.0, 25.5 and 26.7 °C water temperature, and 0.38 and 0.86 mS/cm electrical conductivity (EC), respectively (**Table 11**). In both experiments, DO levels were well above the tolerance limits of 6 mg L^{-1} suggested for fish production (Graber and Junge, 2009). EC levels of two aquaponic treatments in experiment 2 were nearly 2-fold higher than those in experiment 1. This reflected that experiment 2 was conducted consecutively after experiment 1, without diluting the aquaponic solution.

In both experiments, DO, pH, and water temperature were similar among aquaponic treatments (**Table 11**); however, EC tended to be higher (experiment 1) or was significantly higher (experiment 2) in AUF than in AIF. HYD showed the highest DO and EC, but the lowest pH and water temperature among the treatments. Although the amount of pH correction solution used for the one-month period was not significantly different between AIF and AUF in both experiments, the amount tended to be lower in AUF than in AIF.

Average ambient temperatures and vapor pressure deficit were similar between experiment 1 and 2 (23.2 and 23.3°C; 1.7 and 1.7 kPa, respectively) (**Figure S2**). Daily light integral (DLI) was higher during experiment 2 (12.2 mol m^{-2} day^{-1}) than experiment 1 (9.1 mol m^{-2} day^{-1}). There was a tendency for higher cumulative water consumption during experiment 2. Water consumption was significantly higher in aquaponics than in hydroponics. AIF consumed nearly three times higher water than HYD to produce crops, while AUF used 2-fold higher water than HYD (**Table 11**).

Although there was no significant difference in average EC between AIF and AUF in experiment 1, it was significantly (P < 0.05) higher in AUF than in AIF in experiment 2. The EC levels were, however, significantly lower than that in HYD. The EC in two aquaponic treatments gradually increased over time. In experiment 2, EC in AIF increased by 2.7 times (from 0.37 to 1.01 mS/cm) during one-month production, while that in AUF increased by 3.3 times (from 0.36 to 1.20 mS/cm).

3.2. Plant growth and yield; fish growth

Overall plant growth parameters were affected by crop type and nutrient management regime in both experiments (**Table 12**). In experiment 1, plant height and SPAD value were increased remarkably by AUF compared to AIF, whereas most of the other growth parameters were not affected by the feeding regime. Plants showed somewhat intermediate responses to AMF in comparison to AUF and AIF.

While plant growth was slightly reduced in experiment 2 due to the different growing season and crop species (**Table 10**), the results showed similar trends with experiment 1. Compared to hydroponics, growth parameters of vegetables and herbs were reduced by aquaponics regardless of feeding regime. However, there was a trend that AUF increased or tended to increase some growth parameters such as plant height (in leafy vegetable), leaf number (in herbs), and leaf length and area (in leafy and fruity vegetables) similar to those in HYD. Consistent with experiment 1, SPAD values overall increased in AUF compared to AIF regardless of crop type (**Table 12**). Leaf temperatures of crops were significantly higher (in herbs) or tended to be higher (in leafy and fruity vegetables) in aquaponics than in hydroponics.

Similarly, crop yield was affected by crop type and nutrient management regime in both experiments (**Table 13**). Shoot fresh weight showed increasing trends by AUF in both experiments. In general, shoot fresh weights of vegetables and herbs in experiment 2 were smaller compared to experiment 1 due to the different growing season and crop species (**Table 11**).

It was consistent in both experiments that there was an increasing trend of fresh and dry weights of vegetables and herbs grown in AUF than in AIF, especially in experiment 2, to the levels of those in HYD. Particularly, shoot fresh weight (or marketable yield) of Chinese cabbage, mizuna, and Swiss chard grown in AUF significantly increased or tended to increase by AUF compared to AIF to that in HYD (**Figure 7**).

Root fresh and dry weights were lower in AIF than those in HYD (**Table 13**). Compared to AIF, AUF significantly increased (experiment 1) or tended to increase root fresh and dry weights (experiment 2). Crop type and management regimes had significant effects on root growth parameters of vegetables and herbs (**Table 14**). There were interactions ($P < 0.001$) between crop type and nutrient management in root characteristics including root length, surface area, volume, and average diameter. Regardless of crop type, plants grown with AIF had considerably reduced root length, surface area, and average diameter compared to those with HYD. However, AUF significantly increased or tended to increase one or more of these root parameters similar to HYD. Root parameters of leafy vegetables were least affected by AUF. Root diameter class analyses showed that fine root (0.0 to 0.25 mm) growth was generally inhibited by aquaponics and AUF promoted fine root growth only in fruity vegetable (**Table 14**).

Table 9 showed initial fish biomass, feed conversion ratio (FCR), and fish biomass increment in experiment 1 and 2. AIF had lower feed conversion ratio (total feeding amount divided by fish biomass) and higher fish biomass increment than AUF in both experiments.

3.3. Nutrient concentrations and dynamics in aquaponic solution as affected by management regime.

The average mineral nutrient (NO_3–N, NO_2–N, NH_4–N, PO_4–P, K, Ca, Mg, SO_4–S, Na, and Cl) concentrations in the aquatic phase of AIF, AUF, and HYD during the experiment period were presented in **Table 15**. Concentrations of NO_3–N, NH_4–N, PO_4–P, Ca, Mg, and SO_4–S were significantly higher in HYD compared to AIF with the exception of NO_2–N and Na. However, AUF significantly ($P < 0.001$) reduced the average concentrations of NO_2–N and Na compared to those in AIF. Meanwhile, the average concentration of K in AIF was as high as that in HYD averaging at 226 mg L^{-1}, which was reduced to 162 mg L^{-1} in AUF but to a sufficient level for plant growth.

Figure 8 shows the dynamic changes of macronutrients in the aquatic phase as affected by aquaponic management regimes, AIF and AUF, in comparison to HYD. The concentration of all nutrients increased over time with a similar pattern between AUF and AIF in most nutrients.

However, the concentrations of NO_3–N, PO_4–P, Ca, and Mg tended to be highly maintained in AUF for nearly a week compared to those in AIF (**Figure 8 A, D, G, and H**). K levels were consistently higher in AIF (**Figure 8 F**), since the amount of KOH to raise pH tended to be higher in AIF due to larger pH drops (**Table 11**). Na and Cl concentrations were also highly maintained in AIF than AUF throughout the study period (**Figure 8 I, J**).

3.4. Total N concentration in plant tissues and N use efficiency

Total N concentrations varied greatly by crop type and management regimes and were significantly lower in the shoots of crops grown in AIF than those in HYD (**Table 16**). AUF significantly increased or tended to increase total N concentrations in leaves and/or fruits, and therefore, total N concentrations of entire plants were significantly increased by AUF to a similar or slightly lower level to those in HYD. Such an increase in total N concentrations was observed in all crop types. Higher yield was achieved in AUF with the same amount of N applied (**Tables 9 and 13**), and therefore, nitrogen use efficiency (NUE) was significantly higher in AUF, followed by AIF and HYD regardless of crop type.

3.5. Photosynthetic properties

There were significant ($P < 0.01$) differences in photosynthetic parameters among the treatments at Day 7 after transplanting, which include photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and intercellular CO_2 concentration (Ci) (**Table 17**). Regardless of crop type, vegetables and herbs grown in AIF had significantly lower Pn and gs compared to those in AUF and HYD. Similarly, E was lower in crops in AIF than that in HYD, and AUF increased E in leafy and fruity vegetables. The Pn, gs, and E were either weakly or not correlated with leaf temperature (data not shown).

There was an interaction ($P < 0.0001$) between management regime and crop type on intrinsic water use efficiency (WUE) (**Table 17**). Overall, intrinsic WUE of herbs and fruity vegetable was significantly higher in AUF followed by HYD and AIF. Contrarily, intrinsic WUE of leafy

vegetables was higher in HYD than those in AIF and AUF, and not affected by feeding regime. **Figure 9** showed weekly changes in *Pn* of crop species as affected by feeding regime treatment. While there were slight variations among the crops, the overall photosynthetic rate was the highest in HYD followed by AUF and AIF from Day 7 after transplanting and this trend was consistent until the end of the study. Regardless of management regime and crop type, *Pn* considerably increased at Day 14 and decreased at Day 21, except for pac choi, of which *Pn* further increased at Day 21. AUF maintained higher *Pn* at Day 21 in most crop species tested in comparison to AIF.

4. Discussion

4.1. Increasing feeding regime induces more dramatic changes in pH than uniform feeding regime

Water quality is a primary consideration for aquaponic crop production, especially in a recirculating aquaponic system. Deterioration of water quality parameters not only affects fish physiology, growth rate, and feed efficiency (Yildiz et al., 2017), but also affects plant crop performance, quality and/or yield, and N use efficiency as demonstrated in our study.

Consistent with our previous study, DO levels decreased by 20% in aquaponics treatments due to the oxygen demands of fish and microbes; however, feeding regime did not affect the DO level. Regardless of management regimes, DO in aquaponics averaged at 7 mg L^{-1}, which was well above the tolerance limits of 6 mg L^{-1} (Graber and Junge, 2009) and 30% higher than 5 mg L^{-1}, which is a suggested DO level for aquaculture (Boyd, 1982). Considering that nitrifying bacteria have an optimum range of DO (4 to 8 mg/L) to promote nitrification process (Tyson et al., 2008), the DO levels in our study were sufficient in aquaponic systems.

The EC levels increased linearly from 0.10 to 0.66 mS/cm and from 0.51 to 1.18 mS/cm in experiment 1 and 2, respectively (data not shown). Despite the higher EC, the yield of crops was lower in experiment 2 (**Table 13**), which was partly due to the lower DLI and slower seedling growth (**Figure S2**). The addition of basil, a slow-growing plant, also decreased the average fresh and dry weights of herbs in experiment 2. However, the yield of individual crop species showed similar trends in both experiments where AUF consistently produced a higher yield of vegetables and herbs compared to AIF (**Table 13**).

During our study period, pH was measured daily prior to feeding and corrected using a combination of base solutions (1N KOH: 0.05N Ca(OH)$_2$=1:1 (v:v)) in AIF and AUF to be maintained at around 7, which is generally considered an optimum pH value for aquaponics environment (Tyson et al., 2008). Therefore, the pH changes recorded in our study were considered mainly due to the differences in water chemistry affected by the treatment. The average values of pH did not show the daily differences in pH between AIF and AUF due to elapsed time intervals between the discontinuous data points. However, the amount of pH correction solution used for the one-month period consistently tended to be higher in AIF by 20% (**Table 11**). Consequently, such differences affected K concentrations in aquaponic solution, resulting in a 40% higher accumulation of K in AIF than in AUF (**Table 15**). These results indicate that AIF undergoes more dramatic changes in pH than AUF. It is known that carbon dioxide as a result of fish respiration

directly affects the overall system performance by decreasing the pH, which can stress the fish and inhibit the nitrifying bacteria in the biofilters (Ebeling and Timmons, 2012). Therefore, the tendency of lower pH in AIF may be partly due to a higher release of carbon dioxide from increased respiration of fish in the system derived from more active growth. In fact, fish biomass increment consistently tended to be higher and FCR ended to be lower in AIF in both experiments, compared to other feeding regimes (**Table 9**). These results indicate that AIF is more desirable for fish production rather than simultaneous production of fish and plant crops in aquaponics. In fact, plant crop yield and quality were significantly reduced by AIF, and this aspect will be discussed further.

4.2. Aquaponic uniform feeding increases mineral nutrient availability and reduces NO_2–N and Na concentrations for better seedling establishment.

In our previous study, we reported that average concentrations of NO_3-N, PO_4-P, Ca, and Mg were significantly lower in aquaponics than in hydroponics (Chapter 2, this dissertation): 161 and 200 mg L^{-1} NO_3-N, 27 and 126 mg L^{-1} PO_4-P, 20 and 149 mg L^{-1} Ca, and 2.4 and 39.0 mg L^{-1} Mg for aquaponics and hydroponics, respectively, during a 3-month production period. Our present study showed a similar trend that aquaponic solution had lower concentrations of these mineral nutrients compared to the hydroponic solution; however, AUF significantly reduced the average concentrations of toxic compounds (NO_2-N, NH_4-N, and Na) during production period (**Table 16**). Further, there was a clear tendency that AUF increased initial concentrations of mineral nutrients (i.e., NO_3-N, PO_4-P, Ca, and Mg) but decreased concentrations of toxic compounds (i.e., NO_2-N and Na), although the concentrations were not statistically significant ($P > 0.05$) between AIF and AUF during the first week after transplanting (**Figure 8**). This is probably due to elapsed time intervals between the discontinuous data points in nutrient concentrations. There is no doubt that continuous exposure to different water environment for nearly a week made a substantial impact on the seedling establishment and recovery from transplanting stress. It is likely that improved water chemical properties in AUF might have allowed faster recovery of seedlings from transplanting stress. This postulation can be supported by our results that plants grown in AUF had significantly higher photosynthetic rates at Day 7 and throughout the production period (**Table 17**; **Figure 9**). Further, plants in AUF had somewhat increased root growth compared to those in AIF (**Tables 13 and 14**). Seedlings typically undergo transplanting stress immediately following transplanting prior to the establishment of root systems (Kim et al., 2008), which is caused by the disturbance of the functional continuity at the soil-root interface (Sands, 1984). It is well-known that this can lead to reduced plant growth or even plant mortality.

Such transplanting stress negatively affects photosynthetic rate and stomatal conductance (Guehl et al., 1989) and could be substantial when the roots are exposed to the aquaponic solution where water and nutrient environment is suboptimal for seedling growth. It is striking to note that simple modification of feeding regime and subsequent water chemistry changes were effective in improving plant growth and performance in aquaponics.

Nitrification is a biological process that maintains water quality in aquaponic systems by converting a toxic form, ammonia-nitrogen (NH_3–N), into a non-toxic form, nitrate (NO_3–N), to fish and plants in biofiltration units. The intermediate product of nitrification, nitrite (NO_2–N), is also known to be toxic to both fish and plants at low levels. Hoque et al. (2018) informed that the

growth of romaine and iceberg lettuce was reduced by NO_2–N at concentrations as low as 5 mg/L in hydroponic solution. Direct contact with nitrite at this concentration can damage root tips as demonstrated in tobacco (*Nicotiana tabacum* L.) (Hamilton and Lowe, 1981). Our results showed that aquaponics reduced growth of fine roots (0 to 0.25 mm diameter) compared to hydroponics (**Table 14**) even at concentrations below 1 mg/L. It is well expected that NO_2–N concentrations fluctuate more widely in aquaponics than those in hydroponics, especially after feeding, possibly exposing roots to a detrimental level of NO_2–N to root growth. Although AUF maintained fine root growth in tomato plants to the level of hydroponics, fine root growth was consistently reduced in leafy vegetables and herbs grown in aquaponics regardless of feeding regime. These results indicate that a higher level of NO_2–N may be involved in reduced root growth in aquaponics, subsequently affecting crop yield and quality. This aspect needs further investigation.

4.3. Uniform feeding increases quality and/or yield of vegetables and herbs in aquaponics.

Consistently with our previous results (Chapter 2, this dissertation), SPAD value and overall crop yield were lower in aquaponics than in hydroponics when crops were grown with the standard feeding regime (i.e., AIF). However, this study found that AUF not only improved SPAD value in all tested crops grown in aquaponics but also increased yield in leafy vegetables compared to AIF. These results are phenomenal because higher quality and/or yield was achieved by a simple modification of feeding regime even without supplemental nutrients or foliar applications as suggested in other studies (Rakocy et al., 2004; Rakocy et al., 2006; Roosta and Hamidpour, 2011). When basil was grown for 3 months, yield was significantly lower in aquaponics compared to hydroponics (Chapter 2, this dissertation). In this study, total marketable yield of herbs (i.e., basil and chia) was not affected by feeding management regime. The discrepancy is considered due to the duration of studies. As basil grows slowly during the seedling stage, this short-term study may have not differentiated treatment effects. Therefore, the feeding regime is likely to have more significant effects on crop yield if the crops were grown longer term. It should be noted that AUF significantly increased SPAD values in all vegetables and herbs tested in this study, suggesting its potential for practical applications in recirculating aquaponic systems to improve crop quality and yield. Recirculating aquaponic systems are known for lower productivity of both fish and plants in comparison to separate recirculating systems (Chapter 2, this dissertation; Kloas et al., 2015; Roosta, 2014; Wortman, 2015); however, it may be successfully operated without compromising crop yield and quality if the uniform feeding regime is integrated and combined with other production strategies.

Interestingly, total fresh and dry weights of vegetables and herbs grown in AUF were similar to those in HYD, despite the fact that HYD provided more than twice the EC of aquaponics, and at least three times higher concentrations of NO_3–N, PO_4–P, Ca, and Mg than those in AUF (**Tables 11 and 15**). Such results can be explained by the general concept that crops can grow optimally as long as a steady-state concentration of nutrients is maintained (Le Bot et al., 1998). For example, increasing NO_3-N concentration above 1 mM (14 ppm) in nutrient solution did not increase the growth rate of corn, soybeans, sorghum, bromegrass, and ryegrass during the first 3 weeks (Clement et al., 1978; Edwards and Barber, 1976; Warncke and Barber, 1974). Similarly, fertilization of young rice plants above the concentration of 0.9 mM N (13 ppm) at root surface

did not increase crop uptake, leading to inefficient N accumulation by the crop (Youngdahl et al., 1982). The steady-state concentration can be crop specific, and the vegetables and herbs examined in this study may require a higher concentration to ensure optimal growth and yield to the levels of those in hydroponics. In addition, the levels of Ca and Mg in aquaponics were, respectively, six and ten times lower than those in hydroponics despite doubling their initial concentrations via AUF (**Table 15**; **Figure 8**). Liebig's law of the minimum describes plant growth is determined by the least available nutrient in the root medium, and therefore, Ca and Mg may act the least available nutrients that determine crop growth and yield in aquaponics (Chapter 2, this dissertation) and need to be supplemented particularly during seedling establishment in aquaponics.

4.4. Uniform feeding improves total N content and photosynthetic performance of vegetables and herbs in aquaponics

It is striking to find that uniform feeding rate positively affected photosynthetic rate in most crop species from the first week after transplanting, to a similar level as those in hydroponics, and subsequently enhanced plant photosynthetic performance throughout production period (**Figure 9**). Such better performance of plant crops is considered to be associated with better nutrient availability and uptake during the first week of seedling establishment. In fact, the SPAD values of crops in AUF were significantly higher than those in AIF from the 2nd week of study (data not shown), which confirms that higher nutrient availability during the early stage of seedling establishment is critical for crop performance in aquaponics. While AIF is the standard nutrient management regime in aquaponics, it is apparent that this practice has a negative impact on crop photosynthetic performance, by decreasing chlorophyll content and total N content in the leaves. Leaf growth and development is tightly controlled by genetic and environmental factors. The mechanisms that regulate leaf size remain unclear but include spatially and temporally coordinated cell expansion and cell cycle activity (Gonzalez et al., 2010). Young developing leaves are more sensitive to nitrogen fertilizer than mature leaves (Ding et al., 2018) as an increase in cell number is mostly responsible for the expansion of younger leaves (Gonzalez et al., 2010). It is likely that uniform feeding regime promoted leaf total N content and leaf growth, especially during the seedling stage, leading to a better photosynthetic performance of crops and ultimately improving crop quality and/or yield. These results confirm our conclusion that AUF is a more suitable feeding regime for aquaponic crop production, as quality (leaf greenness) and/or yield of plant crops in AUF was consistently higher than those in AIF.

4.5. Uniform feeding is more effective in nitrogen use for plant crop production than increasing feeding in aquaponics.

The information on feeding rates is available for most commonly cultured fish species, and therefore, current nutrient management practices in aquaponics heavily rely on fish feeding practices commonly used for aquaculture (Somerville et al., 2014). Optimum feeding rate in aquaculture ranges typically from 1 to 5% of their body weight per day, and changes depending on the average size in length or weight and the number of fish in the tank, raceway, or pond (New, 1987). For example, the optimum feeding rate of 200g fish (farmed *Tilapia nilotica* at 46% protein commercial fish feed) is 1.8% of biomass per day and reduced to 1.5% (high to low) (New, 1987).

Meanwhile, the feeding rate commonly suggested in a recirculating aquaponic system is at around 1 to 2% of fresh body weight per day to maintain water quality of recirculating water (Somerville et al., 2014), and this increasing feeding rate can be adjusted based on fish growth rates and appetite to maintain overall system balance.

However, our results demonstrate that increasing feeding not only reduces the quality and/or yield of vegetables and herbs but also decreases nitrogen use efficiency (NUE) of the system. Although NUE was increased by 4 to 6 times in aquaponics compared to HYD even without considering N used for fish, NUE was further increased by over 30% when AUF was employed. These results are significant because a simple change in feeding regime can significantly enhance N use efficiency of aquaponics for the production of vegetables and herbs, which can contribute to the success of recirculating aquaponic systems.

There are two feed input methods recommended by Rakocy (2007) to keep nutrient input to an aquaponic system relatively constant. For a system consisting of multiple rearing tanks, such as UVI system, where tilapia in each tank are at different stages of growth and harvested every 6 weeks, it is recommended to gradually increase feed input to maximum input over 6 weeks and drop by 25 to 30% at fish harvest of one tank and restocking with fingerlings. For a system with one rearing tank, feed input would slowly increase to maximum input over 24 weeks and decline by 90% at fish harvest and restocking. Based on our results, feed input during this production period can be better managed by integrating uniform feeding. A majority of aquaponic farmers tend to operate on a smaller scale (Love et al., 2015). Therefore, in a small-scale operation with one rearing tank, feeding amount for a month should be estimated based on fish size, and daily feeding rate is calculated and maintained for a plant crop production cycle. Once plant crops are harvested, the feeding rate can be increased based on the initial fish weight and maintained during the following month of plant crop production. The guidelines can be further developed for a system with multiple rearing tanks. Implementing such changes in aquaponics operation will greatly enhance N use efficiency of aquaponic systems and be the first step toward successful cash crop productions in recirculating aquaponics.

5. Conclusion

Standard increasing feeding practices (feeding rate at 1% fish body weight) in aquaponics have negative impacts on the quality and yield of vegetables and herbs. Simple modification of the current increasing feeding regime considerably improved quality and/or yield of vegetables and herbs in aquaponics. Uniform feeding regime (application of the same amount of fish feed throughout plant production period) not only enhanced the photosynthetic performance of vegetables and herbs in aquaponics, closely or similarly to those in hydroponics, but also increased leaf chlorophyll content and total N content. Such improvement in crop performance, quality and/or yield was associated with increased availability of mineral nutrients during seedling establishment and decreased concentrations of toxic compounds. This simple but highly effective feeding regime significantly improved the nitrogen use efficiency of aquaponic systems. This is the first time demonstrating nutrient management regime that is more suitable for recirculating aquaponics. Developing production strategies in combination with the uniform feeding regime will

further improve the productivity of aquaponics, leading to successful commercial aquaponic crop production.

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Table 9. Nutrient source and application method in aquaponics and hydroponics used in this study. In experiment 1, three different nutrient management schemes were tested in aquaponics with increasing feeding (AIF), intermediate feeding (AMF), and uniform feeding (AUF). In experiment 2, AIF and AUF were compared with hydroponics (HYD).

| Treatment | Experiment 1 | | | Experiment 2 | | |
| | Aquaponics (AQU) | | | Aquaponics (AQU) | | Hydroponics (HYD) |
	Increasing feeding (AIF)	Intermediate feeding (AMF)	Uniform feeding (AUF)	Increasing feeding (AIF)	Uniform feeding (AUF)	
Nutrition source	Fish feed	Fish feed	Fish feed	Fish feed	Fish feed	Commercial fertilizer
Total feeding amount (g/4weeks)	1800	1800	1800	1800	1800	–
Stocking density (kg/m^3)	19	19	19	25	25	–
Initial fish biomass (g/fish)	183	187	180	240	235	–
Initial feeding amount (g)	40 (0.5%)	50 (0.7%)	60 (0.8%)	40 (0.4%)	60 (0.6%)	–
Final feeding amount (g)	70 (0.8%)	65 (0.7%)	60 (0.7%)	70 (0.7%)	60 (0.6%)	–
Total N applied (g)	120	120	120	120	120	740
FCR[a]	0.9	1.4	1.1	2.1	2.3	–
Fish biomass Increment (%)	27.8	17.4	23	10.5	6.5	–
Suggested feeding rate for aquaculture[b]	(4 reducing to 2%)	–	–	(1.8 reducing to 1.0%)	–	n/a
Suggested feeding rate for aquaponics	(1%)	–	–	(1%)	–	n/a

[a] FCR: Feed conversion ratio. FCR = Total feeding amount (g) / Fish biomass increment (g).
[b] FAO (1987): feeding rate for tilapia (*T. nilotica*) in tanks and cages at 27 to 31°C fed a 46% protein commercial fish feed.

Table 10. Varieties of vegetables and herbs used in the study.

Crop type	Common name	Scientific name	
Leafy vegetable	Chinese cabbage	*Brassica rapa* cv. Tokyo Bekana	Exp. 1 and 2
	Lettuce	*Lactuca sativa* cv. Cherokee	Exp. 1 and 2
	Mizuna	*Brassica rapa* var. *japonica*	Exp. 1 and 2
	Pak choi	*Brassica rapa* var. *chinensis*	Exp. 1 and 2
	Swiss chard	*Beta vulgaris* cv. Rhubarb Chard	Exp. 1 and 2
Herb	Basil	*Ocimum basilicum* cv. Genovese	Exp. 2 only
	Chia	*Salvia hispanica*	Exp. 1 and 2
Fruity vegetable	Cherry tomato	*Solanum lycopersicum* cv. Washington Cherry	Exp. 2 only

Table 11. Water quality parameters, total value of solution used for pH correction, and cumulative water use in aquaponics and hydroponics for one month production period. In experiment 1, three different nutrient management practices were tested in aquaponics with increasing feeding (AIF), intermediate feeding (AMF), and uniform feeding (AUF). In experiment 2, AIF and AUF were compared with hydroponics (HYD).

Experiment	Management regime	DO (mg/L)	pH	Water temperature (°C)	EC (mS/cm)	pH correction solution (mL)	Cumulative water consumption (L)
Exp. 1	AIF	7.6	6.7 ab	25.7	0.35 b	3255	266
	AMF	7.9	6.6 b	25.4	0.40 a	3404	228
	AUF	7.7	6.8 a	25.5	0.39 ab	2728	162
	P	ns	*	ns	*	ns	ns
Exp. 2	AIF	7.0 b	6.9 a	26.9 a	0.82 c	4550 a	290 a
	AUF	7.2 b	7.0 a	26.5 a	0.90 b	3835 a	209 b
	HYD	8.8 a	5.8 c	21.5 c	2.2 a	890 b	119 c
	P	***	***	***	***	***	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the mean of 4 and 6 replicates for experiment 1 and 2, respectively.

ns, *, **, *** mean no significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Table 12. Plant growth parameters of vegetables and herbs grown in aquaponics and hydroponics. In experiment 1, three different nutrient management practices were tested in aquaponics with increasing feeding (AIF), intermediate feeding (AMF), and uniform feeding (AUF). In experiment 2, AIF and AUF were compared with hydroponics (HYD) for plant growth.

	Crop type	Management regime	Plant height (cm)	Leaf number (n/plant)	Leaf length (cm)	Leaf area (cm^2/plant)	SPAD	Leaf temperature (°C)
Exp.1	Leafy vegetable	AIF	24.0 b	22.0	29.8	–	25.3 b	–
		AMF	26.1 ab	27.6	34.0	–	28.7 ab	–
		AUF	27.7 a	23.2	32.7	–	30.1 a	–
			*	ns	ns	–	*	–
	Herb	AIF	48.1 b	83.2	22.9	–	15.3 b	–
		AMF	53.7 ab	77.7	28.1	–	22.6 a	–
		AUF	57.7 a	91.8	26.3	–	26.8 a	–
			**	ns	ns	–	***	–
	ANOVA							
	Crop type		***	***	***	–	**	–
	Management		**	ns	ns	–	**	–
	Crop type × Management		*	ns	ns	–	ns	–
Exp.2	Leafy vegetable	AIF	18.4 b	16.1	24.0 b	1251 b	15.5 c	20.0
		AUF	20.5 ab	17.8	27.5 ab	1847 ab	20.2 b	20.0
		HYD	23.0 a	19.5	29.6 a	2477 a	30.3 a	19.3
			***	ns	**	**	***	ns
	Herb	AIF	32.6	29.6 b	16.5	1836	19.4 b	21.2 a
		AUF	32.9	35.7 ab	16.9	1798	26.0 a	21.4 a
		HYD	35.0	50.0 a	18.9	1889	31.6 a	19.5 b
			ns	**	ns	ns	***	**
	Fruity vegetable	AIF	41.8 b	10.3	38.9 b	5063 b	25.6 b	20.7
		AUF	41.9 b	9.8	42.8 ab	6436 a	31.3 ab	20.8
		HYD	53.2 a	10.0	46.8 a	6195 a	37.5 a	20.1
			**	ns	*	**	***	ns
	ANOVA							
	Crop type		***	***	***	***	***	**
	Management		***	*	***	ns	***	**
	Crop type × Management		*	*	ns	ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 5, 1, and 0 (experiment 1; n=30, 6, and 0 replicates), or 5, 2, and 1 (experiment 2; n=48, 20, and 12 replicates) crop species for leafy vegetable, herbs, and fruity vegetable, respectively.
ns,*,**,*** mean no significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 13. Fresh and dry weights of vegetables and herbs grown in aquaponics and hydroponics. In experiment 1, three different nutrient management practices were tested in aquaponics with increasing feeding (AIF), intermediate feeding (AMF), and uniform feeding (AUF). In experiment 2, AIF and AUF were compared with hydroponics (HYD) for plant growth.

	Crop type	Management regime	Fresh weight (g/plant)				Dry weight (g/plant)			
			Total	Shoots	Roots	Fruits	Total	Shoots	Roots	Fruits
Exp.1	Leafy vegetable	AIF	162.8 b	144.4 b	18.4	–	6.5 b	6.0 b	0.55 b	–
		AMF	198.8 ab	179.0 ab	19.8	–	9.0 ab	8.4 ab	0.64 ab	–
		AUF	244.3 a	221.8 a	22.5	–	10.9 a	10.1 a	0.86 a	–
			*	*	ns	–	*	*	*	–
	Herb	AIF	191.8 b	136.9 b	55.0	–	14.0 b	12.3 b	1.69	–
		AMF	225.0 ab	158.2 ab	79.7	–	16.1 ab	13.3 b	2.79	–
		AUF	281.8 a	204.0 a	73.8	–	20.2 a	17.4 a	2.78	–
			*	*	ns	–	*	*	ns	–
	ANOVA									
	Crop type		ns	***	ns	–	***	***	***	–
	Management		*	**	ns	–	**	**	***	–
	Crop type × Management		ns	*	ns	–	ns	ns	*	–
Exp.2	Leafy vegetable	AIF	112.4 b	100.6 b	11.8 b	–	4.9 b	4.4 b	0.42 b	–
		AUF	148.2 ab	132.3 ab	15.9 ab	–	6.5 ab	5.9 ab	0.55 ab	–
		HYD	195.6 a	175.8 a	23.1 a	–	8.8 a	7.9 a	0.82 a	–
			***	**	***	–	**	**	**	–
	Herb	AIF	74.1 b	53.2 b	20.9 b	–	4.5	3.6	0.84	
		AUF	84.2 ab	52.0 b	32.2 ab	–	5.1	4.1	1.06	–
		HYD	135.9 a	84.2 a	51.8 a	–	6.3	4.9	1.29	–
			*	*	*	–	ns	ns	ns	–
	Fruity vegetable	AIF	422.3 b	308.9 b	109.3 b	8.2 b	26.2 b	21.5 b	4.44	0.6 b
		AUF	452.3 ab	321.0 b	126.7 ab	10.3 b	27.9 ab	22.5 b	4.98	0.9 ab
		HYD	628.2 a	461.0 a	159.5 a	16.8 a	37.1 a	30.8 a	5.65	1.4 a
			*	*	*	**	*	*	ns	*
	ANOVA									
	Crop type		***	***	***	–	***	***	***	–
	Management		***	***	***	–	***	***	***	–
	Crop type × Management		ns	ns	*	–	*	*	ns	–

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 5, 1, and 0 (experiment 1; n=30, 6, and 0 replicates), or 5, 2, and 1 (experiment 2; n=48, 20, and 12 replicates) crop species for leafy vegetable, herbs, and fruity vegetable, respectively.
ns,*,**,*** mean no significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 14. Root growth parameters of vegetables and herbs grown in aquaponics with increasing feeding (AIF) and uniform feeding (AUF) in comparison to hydroponics (HYD).

Crop type	Management regime	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Root average diameter (mm)	Root diameter class (cm)				
						0-0.25	0.25-0.50	0.50-0.75	0.75-1.0	> 1.0
						Relative root diameter class length				
Leafy vegetable	AIF	4154 b	525 b	4.4 b	0.34 a	61.7 b	31.1 a	4.9	0.86	0.52
	AUF	6142 ab	406 b	3.3 b	0.31 a	59.5 b	34.1 a	5.2	0.74	0.48
	HYD	17801 a	1289 a	8.0 a	0.23 b	74.8 a	20.4 b	3.8	0.71	0.29
		***	***	**	***	***	***	ns	ns	ns
Herb	AIF	5295 b	459 c	4.0 b	0.35 a	40.5 b	47.0	11.2	1.62 a	0.70 ab
	AUF	7205 ab	1018 b	9.1 a	0.35 a	38.4 b	47.5	11.5	1.78 a	0.80 a
	HYD	19126 a	1692 a	12.1 a	0.29 b	55.1 a	35.8	7.9	0.94 b	0.29 b
		***	***	***	***	**	ns	ns	**	*
Fruity vegetable	AIF	56895 b	3642 b	26.5 b	0.33 a	36.0 b	44.5 a	14.6 a	3.65 a	1.26 a
	AUF	55649 b	7585 a	59.3 a	0.29 ab	65.7 a	25.3 b	7.3 b	1.41 b	0.27 b
	HYD	79725 a	6650 ab	45.8 a	0.28 b	65.3 a	23.4 b	7.5 b	2.72 ab	1.09 a
		*	*	***	*	***	**	**	*	**
ANOVA										
Crop type		***	***	***	***	***	***	***	***	***
Management		***	***	***	***	***	***	***	***	***
Crop type × Management		***	***	***	***	**	***	***	***	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 5, 2, and 1 crop species for leafy vegetable, herbs, and fruity vegetable, respectively. Each crop species consisted of 30, 12, and 6 replicates.

ns,*,**,*** mean no significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Table 15. Average mineral nutrient concentrations in the aquatic phase of aquaponics with increasing feeding (AIF) and uniform feeding (AUF) in comparison to hydroponics (HYD) during the study period.

Management regime		NO_3–N	NO_2–N	NH_4–N	PO_4–P	K	Ca	Mg	SO_4–S	Na	Cl
		(mg/L)									
Exp.2	AIF	59.5 b	0.85 a	0.58 b	24.7 b	226.2 a	14.1 b	2.3 b	257.0 b	5.8 a	0.0 b
	AUF	62.3 b	0.48 b	0.54 b	24.9 b	161.5 b	17.2 b	2.3 b	313.1 b	3.9 b	0.8 a
	HYD	201.2 a	0.21 c	1.04 a	74.5 a	221.7 a	94.1 a	27.3 a	471.6 a	0.09 c	0.2 ab
	P	***	***	**	***	**	***	***	***	***	**

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the mean of 4 replicates. Each replicate is the mean of 10 samples collected at different dates. ns, *, **, *** mean no significant or significant at P $\leq$ 0.05, 0.01, or 0.001, respectively.

Table 16. Total nitrogen content and nitrogen use efficiency (NUE) of vegetables and herbs grown in aquaponics with increasing feeding (AIF) and uniform feeding (AUF) in comparison to hydroponics (HYD).

Crop type	Management scheme	Total nitrogen (%)					NUE
		Total	Leaves	Stems	Roots	Fruits	
Leafy vegetable	AIF	3.73 c	3.69 c	–	3.77 b	–	30.7 b
	AUF	4.84 b	4.94 b	–	3.76 b	–	39.8 a
	HYD	6.11 a	6.03 a	–	3.90 a	–	8.2 c
		***	***	–	ns	–	***
Herb	AIF	4.39 b	4.18 b	2.75 b	3.48	–	30.3 b
	AUF	4.78 a	4.58 ab	2.72 b	3.61	–	39.4 a
	HYD	4.85 a	5.61 a	4.08 a	3.63	–	6.5 c
		*	*	**	ns	–	***
Fruity vegetable	AIF	2.66 c	2.33 c	1.80 b	3.63	2.76 c	21.9 b
	AUF	3.62 b	3.56 b	1.89 b	3.58	3.27 b	28.9 a
	HYD	5.11 a	5.90 a	3.95 a	3.31	3.88 a	6.8 c
		***	***	***	ns	***	***
ANOVA							
Crop type		***	***	***	ns	–	***
Management		***	***	***	ns	–	***
Crop type × Management		**	**	ns	ns	–	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 5, 2, and 1 (n=30, 12, and 6 replicates) crop species for leafy vegetable, herbs, and fruity vegetable, respectively.

ns,*,**,*** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

NUE: Nitrogen use efficiency. NUE was calculated by the following formula: NUE= $(N_f - N_u/N_a) \times 100\%$, N_f= the total nitrogen accumulation in the final harvest plant tissue (g); N_u= the total nitrogen accumulation in the initial plant tissue (g); and N_a= the quantity of total nitrogen applied (g).

Table 17. Plant photosynthetic rate (*Pn*), stomatal conductance (*gs*), transpiration rate (*E*), and intercellular CO_2 concentration (*Ci*) of vegetables and herbs grown in aquaponics with increasing feeding (AIF) and uniform feeding (AUF) in comparison to hydroponics (HYD). The values presented here are photosynthetic parameters measured at Day 7 after transplanting.

Crop type	Management regime	*Pn* (μmol CO_2 m^{-2} s^{-1})	*Gs* (mol H_2O m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	*Ci* (μmol CO_2 m^{-2} s^{-1})	WUE (μmol CO_2 mmol^{-1} H_2O)
Leafy vegetable	AIF	6.5 c	0.08 c	1.08 b	235.6 ab	6.3 b
	AUF	7.9 b	0.12 b	1.44 a	251.3 a	6.8 b
	HYD	9.9 a	0.20 a	1.55 a	248.3 b	7.5 a
		***	***	***	*	***
Herb	AIF	5.7 c	0.06 c	0.95 b	239.1 c	5.7 b
	AUF	7.5 b	0.10 b	1.15 b	251.9 b	6.3 a
	HYD	10.2 a	0.17 a	1.80 a	275.6 a	6.1 b
		***	***	***	***	*
Fruity vegetable	AIF	8.9 b	0.08 b	1.20 b	180.1 a	7.8 b
	AUF	11.9 a	0.07 b	0.90 c	114.0 b	13.5 a
	HYD	12.3 a	0.11 a	1.51 a	182.0 a	8.5 b
		***	***	***	***	***
ANOVA						
Crop type		***	**	ns	***	***
Management		***	***	***	**	***
Crop type × Management		ns	ns	***	***	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 5, 2, and 1 (n=30, 12, and 6 replicates) crop species for leafy vegetable, herbs, and fruity vegetable, respectively.

ns,*,**,*** mean no significant or significant at P $\leq$ 0.05, 0.01, or 0.001, respectively.

Figure 6. Schematic diagram of experimental units: (A) aquaponic system; (B) hydroponic system.
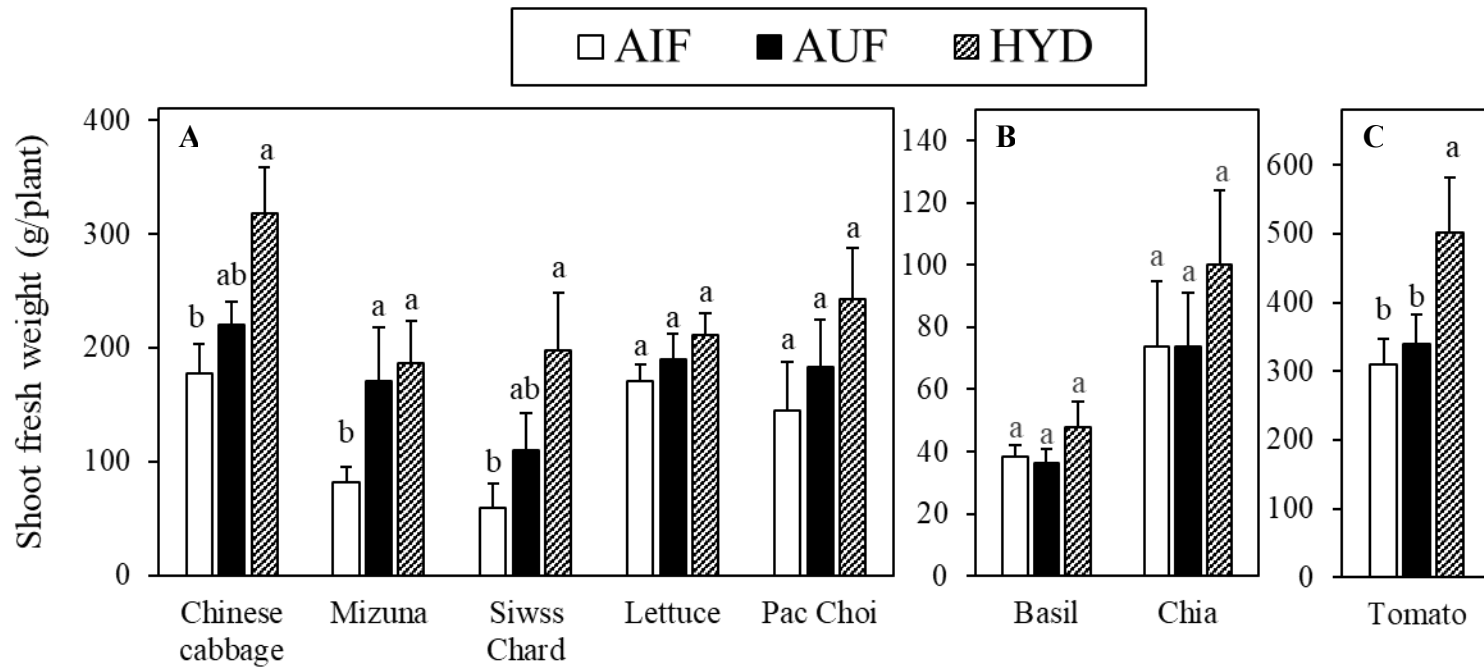
Figure 7. Shoot fresh weight of (A) leafy vegetable, (B) herb, and (C) fruity vegetable grown in aquaponics with increasing feeding (AIF) and uniform feeding (AUF) in comparison to hydroponics (HYD). Data are means ± SE for 6 replicates of each crop species.
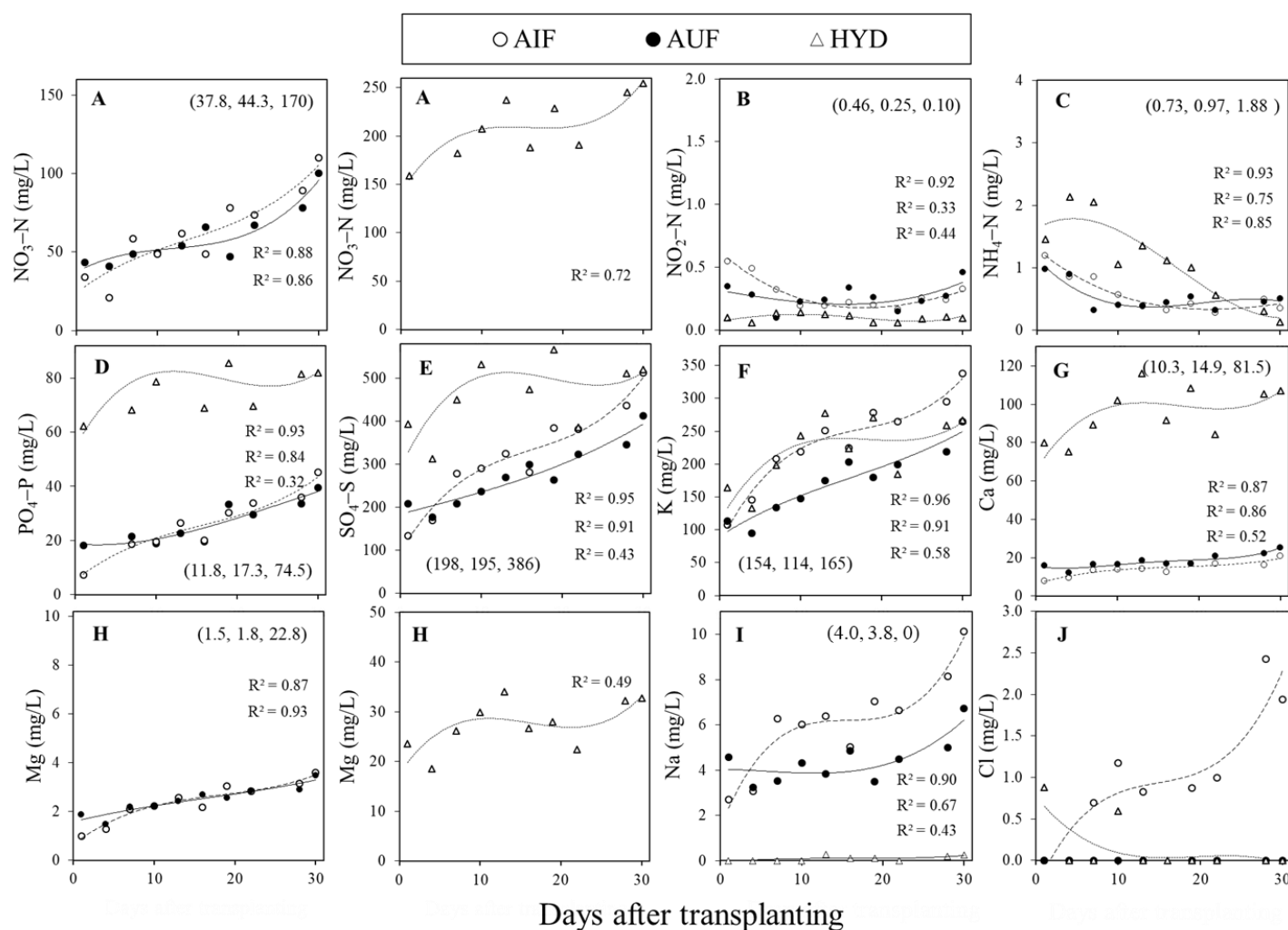
Figure 8. Dynamic changes in macronutrient (A–J) levels as affected by two aquaponic management schemes, increasing feeding (AIF) and uniform feeding (AUF), in comparison to hydroponics (HYD). Water samples were collected once every two days at two different locations in the system (aquaponics: fish tank and hydroponic culture unit; hydroponics: nutrient reservoir and hydroponic culture unit), analyzed using an ion chromatography, and averaged per replicate. Average concentrations of (A) NO_3-N, (D) PO_4-P, (G) Ca, and (H) Mg in AIF, AUF, and HYD were given within parentheses for initial 7 days. HYD of (A) NO_3-N and (H) Mg were presented separately from AIF and AUF of those due to a different scale. Each data point is the mean of 4 replicates.
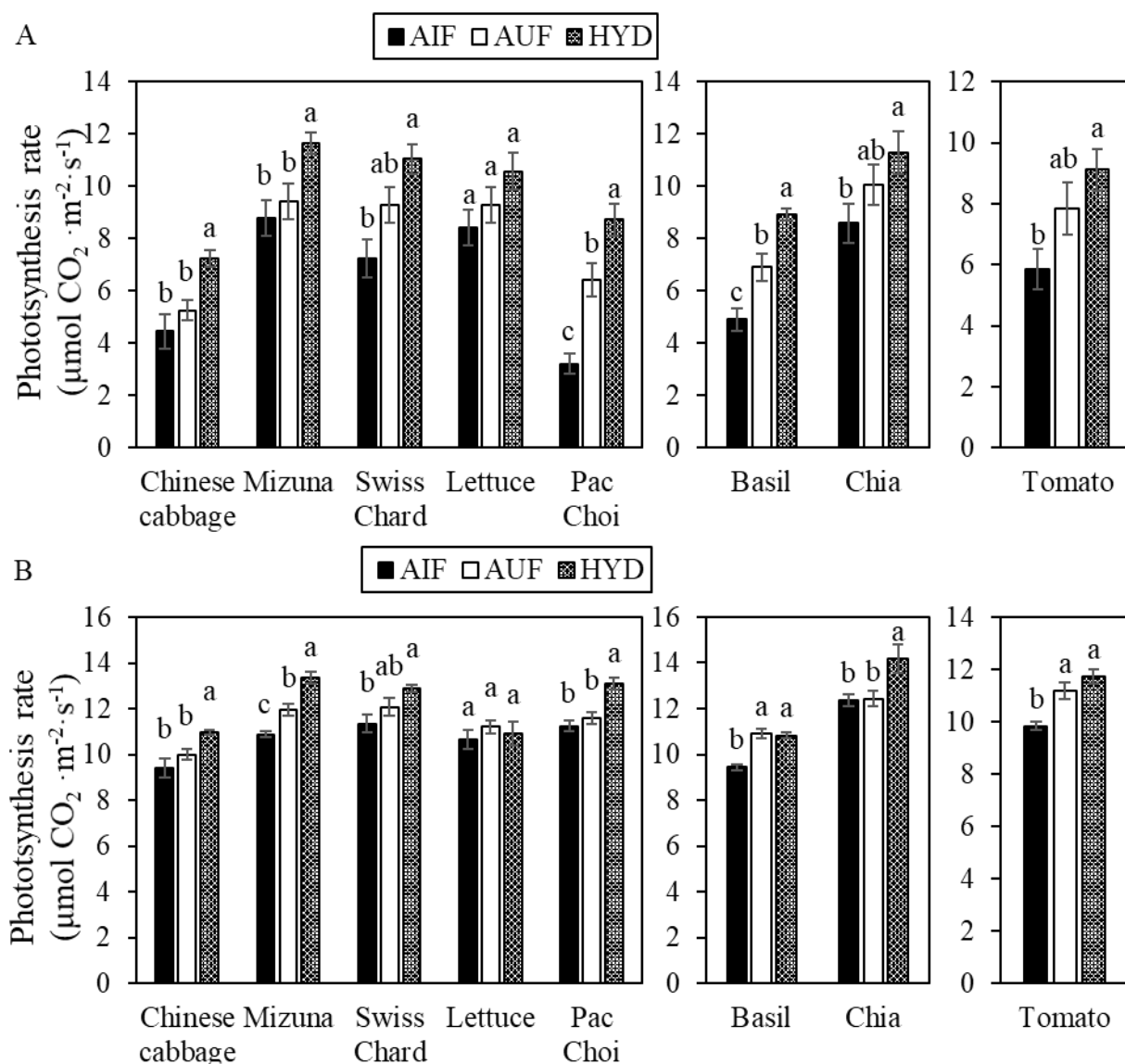
Figure 9. Dynamic changes in photosynthetic rates (*Pn*) of crop species at (A) Day 7, (B) 24, and (C) 21 after transplanting as affected by two aquaponic management regimes, increasing feeding (AIF) and uniform feeding (AUF), in comparison to hydroponics (HYD). Data are means ± SE for 6 replicates of each crop species. Lower case alphabet letters (a–c) represent significant differences across nutrient regime treatments within the crop species. All statistical comparisons were done using a one-way ANOVA and Tukey's HSD (P ⩽ 0.05).

Figure 9 continued

C

Phototsynthesis rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)

Legend: ■ AIF □ AUF ▨ HYD

Chinese cabbage, Mizuna, Swiss Chard, Lettuce, Pac Choi, Basil, Chia, Tomato
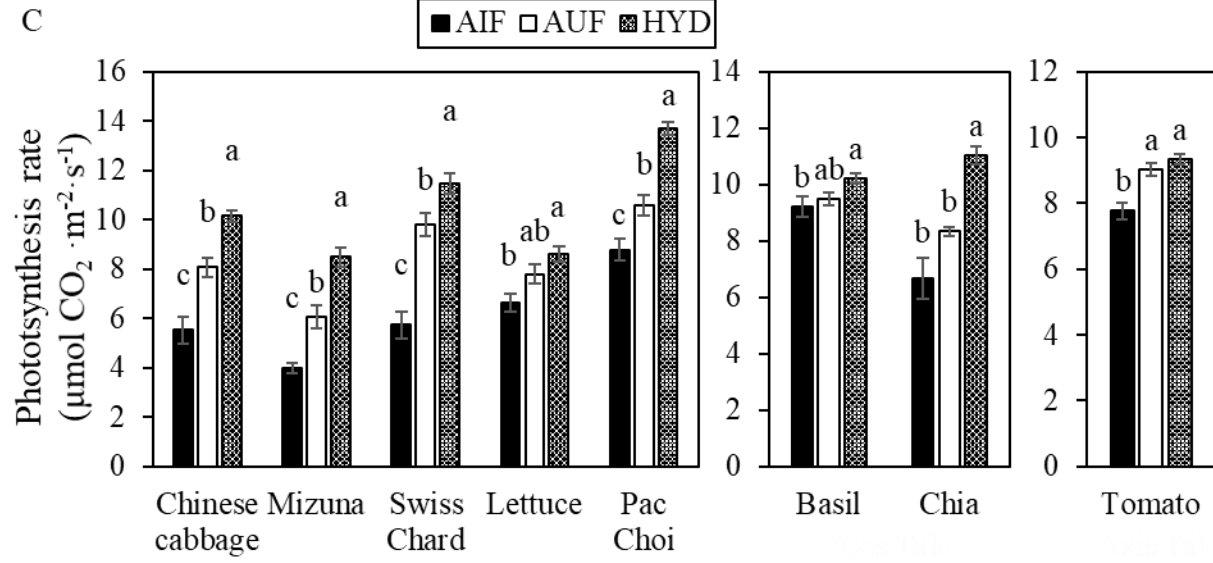
Table S3. Macro- and micro-nutrient compositions and concentrations used in hydroponics and aquaponics.

Parameter	Hydroponics[a]	Aquaponics[b]
Macronutrient (%)		
Total nitrogen (N)	0.043	> 6.88
P_2O_5–P	0.093	> 1.10
K_2O–K	0.035	0.99
SO_4–S	–	0.43
Ca	0.075	2.25–2.75
Mg	0.039	0.23
Micronutrient (mg kg^{-1})		
B	2.00	–
Cu	1.05	10
Fe	21.00	40
Mn	1.90	80
Mo	0.42	–
Zn	2.10	153

All the information come from related company.

"–" means "not contain" or no related information.

[a] Nutrient compositions of fertilizer used in hydroponics were calculated based on 1:100 dilution of commercial fertilizer.

[b] Nutrient compositions of fish feed used in aquaponics were calculated based on g feed per day.
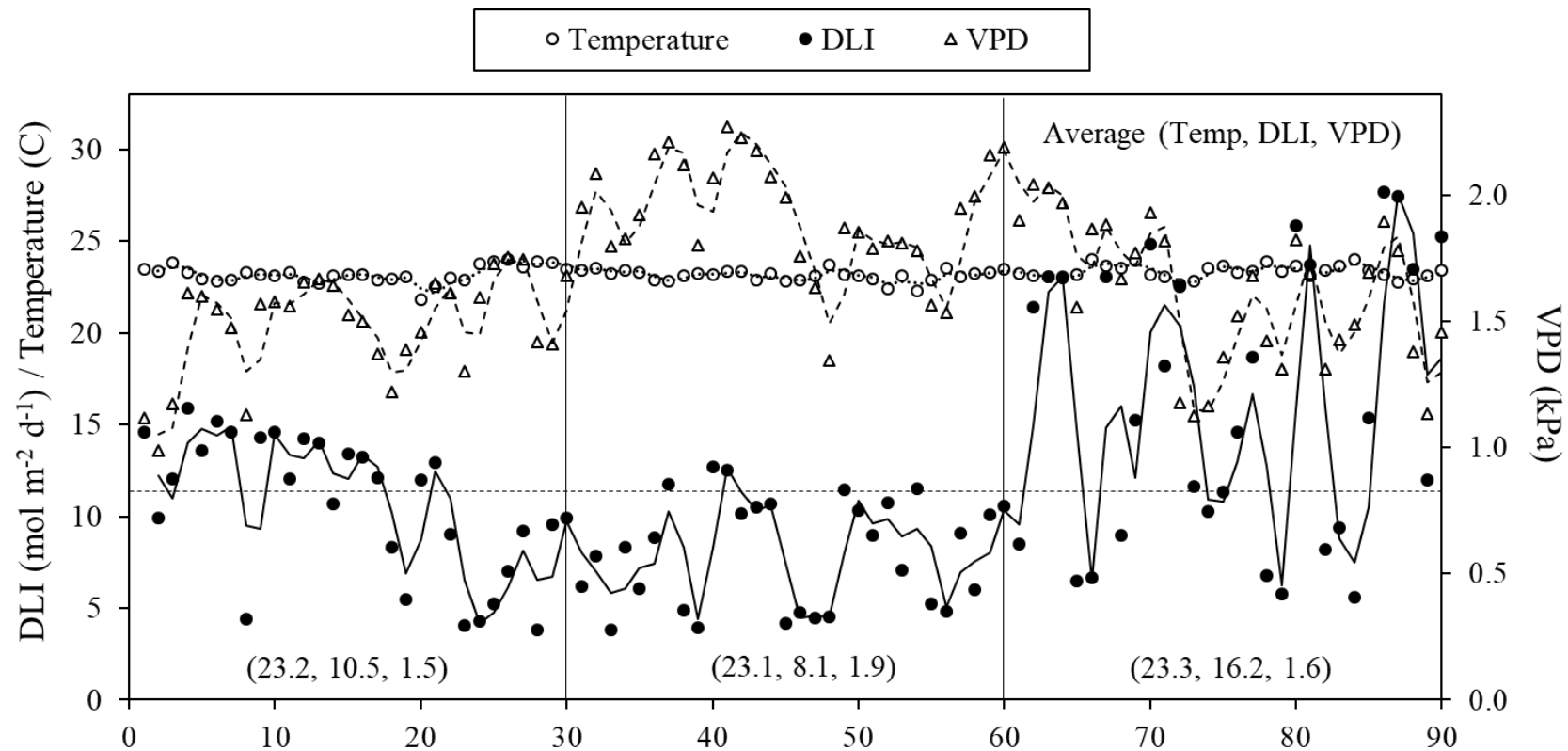
Figure S2. Ambient temperature, daily light integral (DLI), and vapor pressure deficit (VPD) collected in the greenhouse during the experimental period (November to April). The parameters were averaged over the day. A dotted line is the average DLI during entire production period (11.6 mol m^{-2} d^{-1}).

EFFECTS OF HYDRAULIC LOADING RATE ON SPATIAL AND TEMPORAL CHANGES IN WATER QUALITY AND CROP GROWTH AND YIELD IN AQUAPONIC SYSTEMS

Abstract

Aquaponics is a rapidly growing food-production system integrating aquaculture and hydroponic crop production through recirculation of wastewater. Crop performance and yield in aquaponics can be affected by available and harmful nutrients in the root zone, which can be influenced by the flow rate of aquaponic solution. This study was conducted to examine the effects of hydraulic loading rate on water quality and crop performance and yield in recirculating aquaponic systems set at three different loading rates: high (3.3 m/day; HFR, 12 times lower than recommended loading rate), medium (2.2 m/day; MFR), and low (1.1 m/day; LFR). Crop species of varying growth rate were examined: fast-growing Chinese cabbage (*Brassica rapa*) and lettuce (*Lactuca sativa*); medium-growing mustard (*Brassica juncea*) and chia (*Salvia hispanica*); and slow-growing basil (*Ocimum basilicum*) and Swiss chard (*Beta vulgaris*). Water-quality parameters including pH, electrical conductivity (EC), total ammonium nitrogen (TAN), nitrite (NO_2–N), nitrate (NO_3–N), and phosphate (PO_4–P), and plant parameters including leaf greenness (SPAD value), leaf temperature, and photosynthetic rate (*Pn*) were measured regularly. Compared to LFR, HFR significantly lowered the average water temperature while maintaining lower TAN and higher DO and pH during the first 2 weeks after transplanting. HFR decreased the exposure duration of roots to NH_3–N by rapidly dissipating NH_3–N and reducing its peak concentration by 90% and 40% compared to MFR and LFR, respectively, while increasing NO_3–N concentration by 50% and 80%. Lower EC and lower concentrations of NO_3–N, PO_4–P, and SO_4–S in HFR during the last two weeks of production was associated with higher growth rate and total fresh (FW) and dry (DW) weight of plant crops, more prominently in shoots than roots. The leaf greenness (SPAD value), photosynthetic rate (*Pn*), and total N of crops were also significantly higher at HFR than LFR. Fish growth rate, biomass, and feed-conversion efficiency were also increased by HLR. The growth (total fresh weight, shoot fresh weight, leaf area and photosynthetic rate) of fast-growing crops was improved by both HFR and MFR compared to LFR, while those of slow-growing crops were improved only by HFR, indicating that plant-growth rate should be taken into consideration in determining the flow rate. In conclusion, flow rate is an important component for aquaponic crop production as it affects spatial and temporal water quality and subsequently determines the growth and yield of the crops.

1. Introduction

With rapid population growth and growing scarcity of prime cropland, water, and fossil-fuel energy, providing sustainable solutions for food production has become a major challenge. There is a strong need for highly productive and sustainable food-production systems, while maximizing water and nutrient reuse and reducing impact on the environment, especially in areas where water resources are limited (Yang & Kim, 2019). As an integrated system of hydroponics with

aquaculture, aquaponics holds great promise for helping to ameliorate the challenges associated with crop production through efficient use of resources, in which ammonia (NH_3–N), a harmful compound contained in wastewater from fish cultivation, is converted into nitrate (NO_3–N), a nutrient essential for plant growth, by biofilter microorganisms. Plant uptake of the products allows reclaimed water to flow back to the fish tank (Rakocy, Hargreaves, & Bailey, 1989). Recirculating aquaponic systems (RAS) have gained popularity in recent years, which is considered an important driver for the development of integrated food-production systems. About 74% of aquaponic systems are indoor-based, recirculating systems where water is continuously recycled through an interconnected series of fish tanks and crop hydroponics (Love et al., 2014). More than 83% of commercial aquaponics are small-scale systems which contain aquaponic units with a fish tank size of about 1000 L and growing space of about 3 m^2 (Somerville, Cohen, Pantanella, Stankus, & Lovatelli, 2014). Small-scale aquaponic systems are typically characterized by higher water-use efficiency but lower electrical-use use efficiency (S. E. Boxman, Zhang, Bailey, & Trotz, 2017; Love et al., 2014). Well-managed aquaponics could not only reduce water usage and waste discharge to the environment but also improve nutrient retention efficiency and energy efficiency.

Aquaponics can be considered a wastewater-treatment system as it involves nitrification and removal of organic solids or biochemical oxygen demand (Graber & Junge, 2009). The operational conditions of municipal wastewater treatment systems have been well examined (Klinger & Naylor, 2012; Longo et al., 2016; Sun et al., 2016; Q. H. Zhang et al., 2016), and therefore the conditions for an aquaponic system could be handled in a similar manner to improve its performance. Physical and biological processes of wastewater-treatment systems are known to be influenced by pH, dissolved oxygen (DO), and hydraulic retention time (HRT), and therefore these parameters should be maintained within optimal ranges in order for biological nitrification-denitrification processes to occur. Municipal wastewater should be maintained within the optimal pH range of 7.5 and 8.6, DO greater than 1.0 mg/L (Tchobanoglous & Burton, 1991), and flow rate at 108-216 L/day (Chen & Chen, 2000).

As an energy-demanding process, water pumping and recirculation rate are integral parts of aquaponic operation. Water-flow rate is considered one of the key operating parameters of water-based production systems as it affects influent and effluent characteristics, and therefore determines flux of nutrients to the root zone. High flow rates allow faster transport of water and nutrients to the root zone in growing media (Caron, Riviere, Charpentier, Renault, & Jean-Charles, 2002), hydroponics (De Swaef, 2011; Raviv, Wallach, Silber, Medina, & Krasnovsky, 1999), aquaculture wastewater (Lin, Jing, Lee, & Wang, 2002), and municipal wastewater treatment (Allaire-Leung, Gupta, & Moncrief, 2000; Caron et al., 2002; De Swaef, 2011; Mamat et al., 2017; Raviv et al., 1999). For example, when nutrient film technique (NFT) was used as a delivery system of hydroponic solution, water flow rate as high as 1 to 2 L/min (2,500 to 3,000 L/day) accommodated water and nutrient demand of plants and increased biomass of many crops by 10 to 30% including lettuce (Al-Tawaha et al., 2018; Khater & Ali, 2015), chrysanthemum (Blok, Jackson, Guo, de Visser, & Marcelis, 2017), tomato (De Swaef, 2011), and ornamental plants (Jie Xu, Mancl, & Tuovinen, 2014). However, research on water flow rate optimization in deep water culture (DWC) has been limited due to more research focus on its highly dependent dissolved oxygen (Al-Tawaha et al., 2018). Because as a floating system, plant roots in DWC are submerged

in water and not soil (which has gaps and holes where air resides), oxygen is generally considered as the limiting factor for better yield.

High water-flow rate (HFR, L/day) can be translated into high hydraulic loading rates (HLR, $m^3/m^2/day$ or m/day; flow rate/total surface area) or low hydraulic retention time (HRT, h; (surface area $\times$ water depth)/flow rate), although these terms are often used in a similar context. A wide range of HLRs from 0.458 to 1.95 m/day have been used successfully to treat aquaculture wastewater using constructed wetlands (Lin et al., 2002), where NO_3–N removal efficiency was reduced at HLR as low as 0.633 m/day (Lin et al., 2005; Jiabo Xu, Shi, Zhang, Liu, & Zhu, 2014). However, it remains in question if such low HLR has a similar effect on the removal efficiency in aquaponic systems. In contrast, shorter HRT (less than 0.5 h; HLR: 47.8 m/day) has been adopted in conventional aquaponic systems for better fish and plant growth (Shete et al., 2016; Somerville et al., 2014). Water flow has a direct impact on DO and the accumulation of wastes in the fish tank (Somerville et al., 2014) including harmful N species (Endut, Jusoh, Ali, Wan Nik, & Hassan, 2009), and therefore, a HRT as high as 0.5 h (HLR: 47.8 m/day) was recommended in small-scale densely-stocked aquaponic systems to keep all organisms alive, which can be further increased to 1.0 h (HLR: 23.9 m/day) in aquaponic systems with low stocking densities (Somerville et al., 2014). However, such high flow rate is energy-intensive and may not be necessary as long as the water environment is favorable for nitrification and crop growth. Flow rate may influence water environment by rapidly removing harmful compounds but may reduce the contact time of plant roots with beneficial nutrients. In order to enhance crop growth and yield and minimize pumping requirement, the optimal water flow rate of an aquaponic system should be determined.

Meanwhile, the choice of plant species is considered one of the key operational components that can influence the performance of aquaponic systems. For example, plant growth and yield in aquaponics may interact with not only the external conditions, i.e., water quality parameters and nutrient availability, but also the internal factors such as plant growth rate. Therefore, the differences in plant growth rate justify different flow rate to optimize crop production in a water-based system. However, there are limited information on the effects of water flow rate on crop growth with different growth rate.

The main objectives of the study were: (1) to examine the effects of hydraulic loading rate on water quality in aquaponic systems; (2) to investigate the effects of hydraulic loading rate on the growth and performance of crops with different growth rate; and (3) to determine the optimal hydraulic loading rate for efficient aquaponic system for maximum crop yield.

2. Materials and methods

2.1. System design and flow-rate treatment

This study was conducted in the greenhouse at Purdue University at West Lafayette, IN (lat. 40°N, long. 86°W) using six aquaponic systems. Prior to the study, the systems were operated for two months with fish and water in a recirculating system, allowing the biofilter (with microbes) to mature and the nutrient levels to increase for plants. Each aquaponic system was equipped with a

350 L fish tank, a 20 L clarifier, two 20 L biofilter tanks and a 350 L (1.0 m^2) hydroponic culture unit (**Figure 10**). A peristaltic pump (Masterflex, Cole-Parmer, USA) was used to recirculate the aquaponic solution within the system. The total water volume in each aquaponic system was 760 L, and the hydraulic loading rates (HLR) were set at 3.3, 2.2, and 1.1 m/day, respectively, for high- (HFR), medium- (MFR), and low- (LFR) flow rate treatments, giving a water-retention time of 6, 9, and 17 h, respectively, in fish tank and in hydroponic culture unit (**Table 18**). These HLRs were 12 to 35 times lower than the recommended rates by FAO (0.5 HRT) (Somerville et al., 2014) and were chosen based on the flow rates commonly used in hydroponics (Al-Tawaha et al., 2018; Genuncio, Gomes, Ferrari, Majerowicz, & Zonta, 2012). Water in the clarifier captured the majority of suspended solids from the fish tank. After passing through the clarifier, the output solution flowed into the biofilter and then the hydroponic culture unit, where deep-water culture (DWC) was employed. Plants were supported by a foam board set on the top edges of the hydroponic unit. Each of the hydroponic culture units and fish tanks had three air stones to maintain dissolved oxygen (DO) concentrations to nearly full saturation. The fish tanks were covered with a plastic board to prevent algal growth. There was also a lid on each board that remained open to admit light go through plastic tanks during the daytime. Nutrients dissolved in the aquaponic solution were absorbed by plants in the hydroponic culture unit, and the reclaimed water was then recirculated into the fish tank. The water was recirculated continuously within the system and water was not discharged during the study period except for replenishing evapotranspiration losses and fish splashing losses using reverse-osmosis (RO) water. The photoperiod was 14 h consisting of natural daylight with supplemental lighting using high-pressure sodium (HPS) lamps. Day (8:00 am to 10:00 pm) and night (10:00 pm to 8:00 am) temperatures were set at 24 and 18°C, respectively. Supplemental photosynthetic photon flux density (PPFD) in the greenhouse was measured at night using a quantum sensor (LI-250A light meter; LI-COR Biosciences, Lincoln, NE). The average daily light integral (DLI) for both solar and supplemental lighting, temperature, and vapor-pressure deficit (VPD) were 20.3 mol/m^2/d, 23.7°C, and 0.18 kPa, respectively, during the study period (**Figure S3**).

2.2. Plant and fish materials

Nile tilapia (*Oreochromis niloticus*) were obtained from Animal Sciences Research and Education Center at Purdue University, which had been cultivated in a conventional aquaculture system for 4 months prior to the start of the experiment. In each aquaponic system, the fish tank was stocked with a total weight of 7.4 kg fish, equal to a stocking density of 20 kg/m^3, and individual fish weight averaged 284 g. Fish feed used in our study was a complete diet containing 41% protein and 1.1% phosphorus (AquaMax Sport Fish 500, Purina Mills, St. Louis, MO) with 4.8-mm floating pellets. Fish were fed once per day at 9:00 am with feed at an average rate of 0.5% body weight. Aquarium thermostatic heaters (Eheim Jager TruTemp®, Germany) were used to maintain water temperature of the fish tanks within optimum range (27–29°C) for tilapia culture in aquaponics systems. Fish biomass gain was obtained by calculating the difference between initial and final fish weights over the study time (30 days). Feed conversion ratio (FCR), a measure of feed-conversion efficiency, was calculated by dividing the total amount of feed by the fish biomass

gain during the experiment. The specific growth rate (SGR) in grams of fish per day was also calculated by dividing total fish biomass gain by the number of days of the experiment.

There were six different plant species cultured in this study, including Chinese cabbage (*Brassica rapa* cv. Tokyo Bekana), lettuce (*Lactuca sativa* cv. Cherokee), mustard green (*Brassica juncea* cv. Golden Frill), chia (*Salvia hispanica* cv. Red Garnet Microgreens), basil (*Ocimum basilicum* cv. Genovese), and Swiss chard (*Beta vulgaris* cv. Rhubarb Chard). Seeds were purchased from a commercial source (Johnny's Selected Seeds, Winslow, ME) and sown in agrifoam (Syndicate Sales, Inc., Kokomo, IN) trays with intervals of few days in order to match the sizes of seedlings at the time of transplanting. Seeds were initially imbibed with tap water, followed by a half-strength fertilizer solution once germinated, and full-strength fertilizer after seedlings developed true leaves (Kim, Yang, Lin, & Langenhoven, 2018). The seedlings were irrigated as necessary with a combination of two water-soluble fertilizers (3:1 mixture of 15N–2.2P–12.5K Cal-Mag Special and 21N–2.2P–16.6K Multi-Purpose fertilizers, respectively; Everris NA, Dublin, OH). The fertilizer consisted of (mg/L): 150 nitrogen (N), 20 phosphorous (P), 122 potassium (K), 38 calcium (Ca), 15 magnesium (Mg), 0.8 iron (Fe), 0.4 manganese (Mn) and zinc (Zn), 0.2 copper (Cu) and boron (B), and 0.1 molybdenum (Mo). Nitrate form was 76% of nitrogen provided. The pH for the fertilizer was 5.5-6.0. After the third true leaf emerged, uniform, healthy seedlings were randomly chosen and transplanted into mesh pots, each containing 85 g clay balls, then transferred to the hydroponic culture unit of aquaponic systems.

2.3. Plant and fish growth measurements

Crop growth parameters were measured before transplanting and in the fourth week after transplanting, which included plant height, leaf number, and leaf length. Leaf temperature (°C) was measured at the third week after transplanting using an infrared radiometer (MI-210, Apogee Instruments, Inc., Logan, UT, USA) at approximately 4.8 cm away from the leaf surface. The average level of chlorophyll content from each young, fully expanded leaf of the canopy were recorded by taking SPAD (soil plant analysis development) readings with a SPAD-502 Chlorophyll meter (Minolta Camera Co. Ltd., Japan). Five readings per leaf were taken and averaged.

All plant samples were weighed to get an average fresh weight at the beginning, during, and the end of the study. Plant growth rate was calculated by the weight difference divided by the number of days elapsed between the two measurements. At harvest, plant samples were separated into different tissues (roots, stems, leaves), and immediately weighed for fresh weight and all leaf samples were scanned for leaf area using a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA). Harvested samples were oven-dried (over 72 h at 70 °C) until a constant weight was measured for dry weight. Specific leaf area (SLA) was calculated by the ratio of leaf area to leaf dry mass.

At the beginning and end of the study, five fish were randomly selected from each fish tank and weighed to get a five-fish weight, then an individual average fish weight was calculated by dividing total weight by the number of fishes; this procedure was repeated five times to obtain an average individual fish weight for each aquaponic system.

2.4. Water-parameters measurement

The amount of fish feed (g) and water-quality parameters, such as temperature (T; °C), pH, electrical conductivity (EC; µS/cm), and dissolved oxygen (DO; mg/L) at the fish tank and hydroponic culture unit were measured daily before feeding at 9:00 am using the HQ40d portable water quality lab package (HACH Corp., Loveland, CO, USA). A mixture of potassium hydroxide (2 N) and saturated (0.045 N) calcium hydroxide (v:v=1:1) was directly added to the fish tank to adjust pH at around 7.0 daily.

Water samples were obtained from fish tank and hydroponic culture units every 3 days before feeding and were analyzed immediately for TAN, NO_2–N, NO_3–N and PO_4–P concentrations, using HACH reaction kits (Loveland, Co. Ltd., USA), namely Ammonia Reagent Powder Pillows, Nitrite Reagent Powder Pillows, Nitrate Reagent Powder Pillows and Phosphate Reagent Powder Pillows, respectively. Weekly water samples obtained from fish tanks were used to analyze anions (i.e., NO_3–N, NO_2–N, PO_4–P, SO_4–S) using ion chromatography (Dionex ICS–5000, Thermo scientific, Co. Ltd., USA) as described in 2.7.

In the absence of plants, NH_3–N and NO_3–N were monitored *in situ* for 24 h using TruLab pH/ISE 1320P meter (YSI, Inc., Yellow Springs, OH, USA) in the hydroponic tank of aquaponic systems. The pH was manually monitored from 9:00 am to 9:00 pm hourly every day by using the HI9811-5 portable pH/EC/TDS/Temperature meter (Hanna Instruments, Inc., Smithfield, RI, USA). This study was conducted for 7 days and the pH was readjusted to 7 before the application of fish feed at 9:00 am next day.

2.5. Photosynthesis measurements

In the third week after transplanting, four representative plants were randomly selected in each treatment to measure instantaneous photosynthetic rate ($\mu mol/m^2/s$) of their young, fully expanded leaves. Leaf gas-exchange measurements were performed in the greenhouse during daytime hours (between 9:00 am and 2:00 pm) using a portable gas-exchange system (LI-6400XT; LI-COR Biosciences, Lincoln, NE). The leaves were clipped with the 6-cm^2 standard leaf chamber, which was assembled with a light source (LI-6400-02B; LI-COR Biosciences, Lincoln, NE, USA) and designed with red and blue LEDs (665 and 470 nm, respectively). Illumination was supplied at a PPF of 400 $\mu mol/m^2/s$ by the light source under ambient temperature conditions. The reference CO_2 concentration and flow rate through the chamber were 400 µmol/mol and 500 µmol/s, respectively. In each treatment, the measurements of photosynthetic rate (*Pn*), stomatal conductance (*gs*), transpiration rate (*E*), and internal CO_2 concentration (*Ci*) were conducted on each plant of each species between 9:00 am and 14:00 pm. For each plant sample, one leaf at each canopy level was selected for measurement. Readings were taken when the coefficient of variation (i.e., sample CO_2, sample H_2O, and flow rate) was less than or equal to 0.2%, which typically occurred within 10 min. The intrinsic water-use efficiency (WUE) of each leaf sample was calculated by dividing *Pn* by *gs* of that specific leaf measurement. Whole-plant photosynthetic rate was calculated by multiplying the photosynthetic rate (*Pn*) with leaf area as described in section 3.3.

2.6. Total nitrogen measurements

All dried samples were filtered through a 10-mesh sieve after grinding with a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ, USA) and kept in plastic vials for total N analysis. A 30 mg sample was measured and transferred into an empty sample tin using a clean small sampling spatula. The tin was carefully wrapped into a ball and then the total nitrogen content of a sample was measured using the C/N analyzer (FlashEA 1112, Thermo Fisher Scientific, Waltham, MA, USA) as described by Bhattacharyya et al. (2015).

2.7. Anion measurements

For anion nutrient analysis of water samples, the water samples, which were collected and immediately placed in a –20°C freezer, were thawed at room temperature, centrifuged at 12,000 rpm for 10 min, and then liquid supernatants were collected and subjected to anion nutrient measurement. The nutrient compositions and concentrations of processed water samples were analyzed by ion chromatography (Thermo Scientific Dionex ICS–5000, Waltham, US) equipped with anion column (IonPac AS18 column), and a conductivity detector to determine the concentration of anions (including NO_3–N, NO_2–N, PO_4–P, SO_4–S). Chromeleon data management software (version 7.1) was used for data processing.

2.8. Experimental design and data analysis

The experimental design was a randomized complete block design (RCBD) with production system and plant species as the main factors. Six independent aquaponics treatments were operated in each trial (**Figure 10**). The research layout consisted of three flow-rate treatments with two system replicates. Each flow-rate treatment consisted of three sample replicates of six plant species. Four plant replicates were cultured in each aquaponic production unit for each plant species. Each experimental trial was conducted for one month. The experiment was repeated twice from July to October 2017.

All data were statistically analyzed using JMP v13.0 for Windows software (SAS Institute Inc., Cary, NC, USA). The statistical differences were determined using a two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) or Student's t test at a 5% significance level ($P \leq 0.05$).

3. Results

3.1. Physical and chemical parameters of aquaponic solution

Flow rate had no significant effects on the average dissolved oxygen (DO) content of aquaponic solutions (**Table 19**). Similarly, the average pH of aquaponic solution was not significantly different among the treatments; however, it trended lower at low flow rate (LFR) compared to medium (MFR) and high flow rates (HFR). As significantly higher volume of base solution was required for pH corrections in LFR (**Table 19**). Meanwhile, HFR significantly decreased the average water temperature and EC during one-month production period.

Weekly changes in DO, water temperature, EC, and pH indicated that flow rate treatments affected physical and chemical environment of aquaponic solutions (**Figure 11**). Compared to LFR, HFR maintained higher DO and lower water temperature during the first two weeks after transplanting. Contrarily, EC was significantly reduced by HFR during the last two weeks after transplanting. Overall, pH decreased between 1 and 3 weeks but increased by the end of production. HFR showed slower decreasing rate of pH during these weeks, indicating less pH fluctuations in HFR treatment compared to those in MFR and LFR. There were no significant differences among different flow rate treatments in the first two weeks, but HFR showed significantly lower EC than MFR and LFR in the last two weeks.

3.2. N species and other nutrients in aquaponic solution

Flow rate had a negative correlation with the average concentrations of $NO_3–N$ (r= –0.40) and $PO_4–P$ (r= –0.57), suggesting a relatively higher nutrient removal rate by plants at HFR (**Table 20**). These results were consistent with weekly EC data, in which the EC was lower in HFR for the third and fourth weeks after transplanting, indicating that crops grown at HFR remove nutrients at a higher rate or remove them at the same rate, but HFR brings fresh nutrients faster than at slower flow rates balancing the demand for limited nutrients (**Figure 11 C**). Meanwhile, flow rate had a very weak negative correlation with TAN (r= –0.13) and $NO_2–N$ (r= 0.03).

Flow rate treatment affected the concentrations of N species, TAN, $NO_2–N$, and $NO_3–N$ in aquaponic solutions during a 4 week-production period (**Figure 12**). Although the changing trends were similar among the flow rate treatments, HFR consistently reduced $NH_4–N$ concentrations compared to other flow-rate treatments during the entire study period. Although $NO_2–N$ concentration was significantly higher in HFR than LFR in the first week after transplanting, the difference disappeared with increasing weeks of duration. Meanwhile, the concentration of $NO_3–N$ was significantly reduced in the last two weeks compared to those of MFR and LFR.

When dynamic changes in $NH_4–N$ and $NO_3–N$ levels were monitored *in situ* in aquaponic solution for 24 h in the absence of plants, it was found that the $NH_4–N$ spike occurred immediately after the application of fish feed at 9:00 am, and the speed of $NH_4–N$ dissipation was significantly affected by flow rate (**Figure 13**). The highest concentration of the $NH_4–N$ spike varied by flow rate, ranging from up to 100.8 mg/L at LFR to 22.8 mg/L at HFR (**Figure 13**). HFR also increased $NO_3–N$ concentration by 43.9% from 116.8 mg/L to 168.1 mg/L. MFR and LFR also increased the $NO_3–N$ concentrations but to a lesser degree compared to HFR, increasing the concentrations by 21.0% (from 122.6 to 148.4) and 13.1% (from 128.7 to 145.5), respectively. The pH was quickly lowered by HFR compared to MFR and LFR (**Figure 13**), which is most likely caused by a higher nitrification rate as a result of more substrate for the process being delivered to the biofilter in HFR treatment.

3.3. Plant growth and yield

Six crop species were categorized into three groups based on the growth rate (expressed as g total fresh weight increase per day): fast-growing (Chinese cabbage and lettuce), medium-growing (mustard and chia), and slow-growing (basil and Swiss chard) crops (**Figure 14 A**). Regardless of

crop type, the growth rate of crops was reduced by 26.6% when grown at LFR compared to HFR (**Figure 14 B**). The growth rate of fast-growing crops was maintained at MFR to a similar level as that at HFR, while the growth rate of slow-growing crops was significantly reduced at MFR (**Figure 14 B**).

Plant-growth parameters of fast-, medium-, and slow-growing crops at HFR, MFR, and LFR were measured at the end of the study (**Table 21**). Regardless of crop type, plant height and leaf morphological characteristics such as leaf number, area, length, and specific leaf area were not affected by flow rate. However, there were significant morphological differences among crop type. For example, fast-growing crops had significantly higher leaf area but thinner leaves as demonstrated by greater SLA (thinner leaves), but less leaf number than medium- and slow-growing crops. Flow rate significantly affected leaf temperature, and crops grown at HFR treatment had lower leaf temperature regardless of crop type.

Fresh weight of different crop types was affected by flow rate differently. Total, shoot, and root fresh weights of fast- and medium-growing crops increased at both HFR and MFR, while those of slow-growing crops increased only at HFR (**Table 22**). In general, total and shoot dry weight of different types of crops were affected by flow rate in a similar pattern, except roots, for which dry weight was not significantly different among treatments regardless of crop type.

3.4. Fish production

In our experiment, the fish were fed at a constant rate independently of their body mass , in order to maintain water quality and constant nutrient input in the system (Yang & Kim, 2019). Fish stocking at HFR showed the best production performance with the highest fish biomass gain, specific growth rate (SGR), and lowest feed conversion ratio (FCR), but there was no significant difference in fish production (fish biomass gains and SGR) between HFR and MFR treatments (**Table 23**).

3.5. Total nitrogen concentration and SPAD value

Total N concentration of fast-growing crops was more sensitive to water flow rate, demonstrating significant reduction by LFR treatment. The total nitrogen content was the highest in fast-growing followed by medium- and slow-growing crops.

Flow rate significantly affected SPAD value. Regardless of crop type, crops grown at HFR treatment showed higher SPAD value. In addition, SPAD values of fast-growing and medium-growing crops were decreased by 25.3% and 31.3%, respectively, at LFR treatment compared to those at HFR treatment. SPAD value of slow-growing crops at LFR were decreased by 8.9%, compared to that at HFR.

3.6. Photosynthetic parameters

Similarly, flow rate differentially affected total nitrogen content (g/plant) depending on the type of crop (**Table 24**). Crops at HFR treatment showed significantly higher total nitrogen content in shoots, roots and total plant than those at MFR and LFR. Compared to LFR treatment, total

nitrogen content of fast-, medium-, and slow-growing crops in HFR treatment increased by 109.7%, 67.9% and 90.0%, respectively.

Photosynthetic rate, stomatal conductance, and transpiration rate were lower or tended to be lower at LFR regardless of crop type (**Table 25**). In general, flow rate did not have significant effects on intercellular CO_2 concentration and water-use efficiency, particularly in medium- and slow-growing crops. Since the leaf area was greater in fast-, medium-, and slow-growing crops in decreasing order (**Table 21**), net photosynthesis rate, as expressed as the photosynthetic rate multiplied by leaf area, was significantly greater in fast-growing crops (**Figure 15**). On the contrary, net photosynthesis rate was not affected by flow rate in slow-growing crops.

4. Discussion

4.1. Flow rate affects water quality in aquaponic solution

Water flow rate is an important factor that determines water and nutrient availability in the root zone for hydroponic systems (Blok et al., 2017), thus influencing crop growth. However, only limited information is available regarding water flow rate for aquaponic crop production. A wide range of flow rate has been tested for aquaponic crop production (Endut et al., 2009; Shete et al., 2016; Somerville et al., 2014), which was 2 to 12 times higher than what was normally suggested for hydroponic production. Thus, water-flow rate optimization in aquaponics is subjected to closer scrutiny for maximum nitrogen removal and plant production. High flow rate, or low hydraulic retention time (HRT: 0.5 h), has been recommended for aquaponic operations (Endut et al., 2009; Somerville et al., 2014) mainly to avoid the accumulation of total ammonia nitrogen (TAN) and nitrite that are harmful for fish health (Cripps & Bergheim, 2000; Danaher, Shultz, Rakocy, & Bailey, 2013) and to remove nitrite that are is harmful for plant growth (Danaher et al., 2013; W. Li, Liu, Ajmal Khan, & Yamaguchi, 2005; Yang & Kim, 2019).

We demonstrated in this study that hydraulic loading rate (HLR) makes spatial and temporal changes of water physical and chemical parameters in aquaponic solution not only for N species but also dissolved oxygen (DO), water temperature, EC, and pH and flow rate at 3.3 m/day HLR was sufficient for high water quality and aquaponic crop production regardless of crop type, which is 12 times lower than the recommended rate (38.7 m/day HLR; 0.5 HRT). Previous research on life cycle assessment indicated that the electricity required for a water pump is a major contributing factor to the environmental impact in aquaponics, and their impact assessment analysis showed that electricity was a highly sensitive factor and the small reduction on electricity use contributed to a corresponding benefit to the environment (S. E. Boxman et al., 2017). Our study demonstrated that flow rate optimization can benefit the reduction of electricity consumption and present significant contribution to the maximization of the performance of aquaponic systems. We found that low HLR(LFR) at 1.1 m/day (HRT: 17 h) allowed the accumulation of harmful N species in aquaponic solution, negatively affecting the growth and yield of plant and fish crops (**Tables 22 and 23**). Particularly, we found that NH_3–N accumulated up to 100 mg/L, and high levels lasted for extended hours (**Figure 13**), which was also associated with pH changes and nitrification (**Figure 13**). LFR maintained high pH levels in fish tank for extended hours, whereas HFR dropped

the pH to below 7 within an hour. This may explain the reason why a higher volume of pH correction solution was needed to maintain the pH at 7 in LFR treatment (**Table 19**).

Notably, HLR significantly not only reduced water temperature but also decreased leaf temperature of the crops regardless of crop type. In a closed recirculating aquaponic system, the initial amount of energy (thermal energy and kinetic energy) in water is assumed to be the same among different aquaponic systems used in our study. According to energy conservation law (first law of thermodynamics), thermal energy transfers faster into kinetic energy when water moves with higher flow rate (Budiansky & Rice, 1973). This may explain how higher flow rate reduced water temperature of aquaponic solution as observed in our study. We found that crops had lower leaf temperature and higher transpiration and photosynthetic rate when crops were grown at high flow rate (**Tables 21 and 25**). Crop species used in our study were cold season vegetables, which showed higher yield and quality in low air temperature (4.4 – 10 ℃) (Lester, 2006). High flow rate reduced leaf temperature which appeared to have a similar effect as low air temperature, promoting crop yield via increasing photosynthetic rates. Some studies demonstrated the effects of high flow rate on water temperature. For example, Nuwansi et al. (2016) investigated the effect of three different flow rates (0.8, 2.4, and 4 L/min; HLR was unknown) on the growth of water spinach (*Ipomoea aquatica*) in aquaponics systems culturing koi carp (*Cyprinus carpio* var. *koi*) and goldfish (*Carassius auratus*), and observed that water temperature reduced when flow rate increased from 0.8 to 2.4 L/min. However, the result was not conclusive because there was no significant difference between 0.8 and 4 L/min flow rate treatments. (Shete et al., 2016) also observed that water temperature showed a reducing trend when the hydraulic loading rate (HLR) increased from 3 to 12 m/day in an aquaponic system with Common carp (*Cyprinus carpio*) and Mint (*Mentha arvensis*).

The pH change in an aquaponics system is complex and can be caused by multiple factors. Although pH was not significantly different among the flow-rate treatments as a result of pH adjustment (**Table 19**), the pH at LFR in hydroponic culture unit tended to be lower than that at HFR, requiring a higher volume of pH correction solution (**Table 19**). Despite the fact that LFR had slower nitrification and maintained high pH (>7) longer than HFR (**Figure 13**), it remains in question as to the reason for a relatively lower pH trend maintained at LFR (**Table 19**). This may be due to an imbalance of nitrification to crop nutrient uptake as nitrification decreases the pH (Szwerinski, Arvin, & Harremoës, 1986) while plant nitrate uptake increases it (Marschner, Häussling, & George, 1991). In the presence of a high ammonium concentration, continuous loss of cations from the roots of *Pinus sylvestris* seedlings was observed (A. W. Boxman & Roelofs, 1988). The presence of NH_4^+ also reduced Ca^{2+} and Mg^{2+} accumulation in the leaves and the fruit of tomato (*Lycopersicon escuentum* L. cv. Trust F1) and decreased the yield (Siddiqi, Malhotra, Min, & Glass, 2002).

In theory, water-flow rate has an opposite effect on bacterial growth and plant growth. For example, water flow rate as low as 4-day HRT was found to be important for nitrifying bacteria to process NH_4–N to NO_3–N in the municipal wastewater treatment (Anbalagan, Schwede, Lindberg, & Nehrenheim, 2016; Fang et al., 2018). In a study using a constructed wetland to treat aquaculture wastewater, Gao, Li, & Jin (2011) found that a flow rate of 1 m/day HLR (2-d HRT) reduced

contact time of the microorganisms with raw wastewater and roots with partially processed wastewater. In addition, other studies found that higher water flow rate through aquaponic systems also diminished N transformation by the microbial community (Effendi, 2015; Maucieri et al., 2017; Wahyuningsih, Effendi, & Wardiatno, 2015), nitrogen removal rate by plants (G. Li et al., 2018), and plant biomass (Lin et al., 2005; Su, Lin, Jing, & Lucy Hou, 2011). Similarly, Lin et al. (2003; 2005; 2002) and Schulz et al. (2003) examined constructed wetlands to treat recirculating aquaculture wastewater and found that the performance of N removal rate decreased with high HLR ($\geq$ 1.03 m/day).

Too high flow rate may reduce contact time between plant roots and nutrients of the recirculating water. This aspect was demonstrated in some aquaponic studies although direct evidence is still lacking.

For example, high flow rate (HLR: 3.2 m/day) in a recirculating aquaponic system negatively affected N, P and TAN removal rate of water spinach (*Ipomoea aquatica*)(Endut, Jusoh, Ali, Wan Nik, & Hassan, 2010). When a flow-through fish-culture system was used to culture 34 leafy vegetable and herbs, most crop species attained lower biomass at low flow rate (HLR: 1.4 m/day; HRT: 9.9 h) compared with those at high flow rate (HLR: 3.0 m/dayHRT: 2.5 h) (Buzby, Waterland, Semmens, & Lin, 2016), in which aquaponic solution contained extremely low nutrient concentrations (NH_4–N, NO_3–N, and PO_4–P were 0.35, 0.34 and 0.19 mg/L, respectively). Exceptionally high flow rate (77.8 m/day) also did not yield high biomass of lettuce and Swiss chard in aquaponics (Buzby, West, Waterland, & Lin, 2017). Medium flow rate (12000 L/day; HRT: 1.2 h) improved mint growth and yield than high flow rate (24000 L/day; HRT: 0.6 h) (Shete et al., 2016). In a study comparing three flow rates (1.0, 1.5, and 3.2 L/min; 2.5, 1.7, 0.78 h HRT), spinach (*B. vulgaris* var. *bengalensis*) yield increased with increasing flow rate from 1.0 L/min to 1.5 L/min, but decreased when flow rate was further increased to 3.2 L/min (Hussain et al., 2015).

Outdoor pond-scale recirculating systems usually use constructed wetlands to treat and reuse fishpond wastewater (Konnerup, Trang, & Brix, 2011; Lin et al., 2005; Tilley, Badrinarayanan, Rosati, & Son, 2002; S. Zhang, Li, Chang, Li, & Tao, 2014; S.-Y. Zhang et al., 2011), in which much slow flow rate (longer HRT) is needed. Retention time is one of the key parameters influencing the performance of constructed wetlands as a biofilter for aquaculture wastewater treatment (Shpigel et al., 2013). Many studies suggest that the performance of eco-technology is generally a function of HLR, and the selection of proper HLR is required for maximum nutrient remova1 (X.-N. Li, Song, Lu, Xie, & Inamori, 2009) because N transformations by microbes (mainly ammonification, nitrification, and denitrification) can be affected by contact time between microbes and plant biomass (Buhmann & Papenbrock, 2013). This low flow rate seems to work well for a large-scale pond-based recirculating aquaculture system. However, as demonstrated in this study, small-scale recirculating aquaponics presents a unique requirement for flow rate distinctive from large-scale aquaculture wastewater treatment systems.

4.2. Higher flow rate promotes crop growth and yield by facilitating crop nutrient uptake in aquaponic systems

The measurements of dynamic changes in N species as influenced by flow rate showed that the concentrations of $NO_3–N$ increased in accordance with the dissipation of $NH_3–N$ spike. DO increased with HFR, especially during the first two weeks after transplanting (**Figure 11**). These results suggest that HFR may create a water environment for improved microbial activities and therefore promote nitrification. These changes in water physical and chemical environments positively affected plant growth as demonstrated in increased SPAD value, total N content, and whole plant photosynthetic rate, facilitating crops effectively removing nutrients from aquaponic solution through higher biomass production (**Table 19**). Similarly, increase in fresh and dry biomass and fruit number and yield of tomato plants was observed when the flow rate of aquaculture effluent increased from 4 to 6 L/h (0.004 to 0.005 m/day HLR), which was associated with increased plant nutrient uptake of N, P, K, Ca and Mg (Khater, Bahnasawy, Shams, Hassaan, & Hassan, 2015). This range of flow rate is 100 times faster than the loading rate employed in our study (1.1 to 3.3 m/day HLR) further supporting our observations that high flow rate increased nutrient uptake.

Dynamic changes of N species in root environment also somewhat improved root growth (**Table 22**). Higher $NO_3–N$ availability could increase both root production and root nutrient uptake capacity (Drew & Saker, 1975; Lambers, Simpson, Beilharz, & Dalling, 1982). Further investigation about flow-rate-effect on plant root architecture will decipher these results. It has been known that nutrient gradients (physical and metabolic gradients) were essential to sustain primary production in the aquatic environment (Wetzel, 1993). Contrarily, lower flow rate probably limited the transport of N and P to the roots, thus reducing crop growth In fact, it was reported that low flow rate prevented the formation of micro-gradients of nutrients around the roots, where nutrient depletion occurs on a very small spatial scale (Warwick & Hill, 1988).

Crop assimilation is considered the major pathway of N and P removal from wastewater. Our study found that high flow-rate treatment (HLR: 3.3 m/day) achieved not only higher removal rate of N and P and higher plant growth (**Tables 20 and 22**), but also higher fish yield (**Table 23**). When water quality parameters were examined in an aquaponic system with water spinach and African catfish, it was found that a water-flow rate of 1.6 L/min (HLR: 1.28 m/day) gave the best production performance of fish than lower flow rate of 0.8 L/min (HLR: 0.64 m/day) (Endut et al., 2009). However, their study used different size fish among the treatments, so the results cannot be used to fully justify the reason for the improved fish yield at high flow rate. In a study using a small-scale aquaponic system with goldfish and spinach (*Spinacea oleracea*) under four water-recirculation periods (4, 8, 12, and 24 h/day), Shete, Verma, Prakash, Tiwari, & Hussain (2013) found fish production to be highest with higher recirculation periods (12 and 24 h/day) but there were no significant differences in concentrations of TAN, $NO_2–N$, and $NO_3–N$ among the treatments. Similarly, higher total sturgeon weight gain was associated with better lettuce growth under reduced HRT (increased flow rate) from 5.4 to 2.7 min (HLR: 0.02 to 0.05 m/day) (Dediu, Cristea, & Zhang, 2012). Fang et al. (2018) also found increased common carp and microalgae yield when HRT was reduced from 4 days to 0.5 day (HLR: 0.40 to 3.09 m/day).

Although each aquaponic component (fish tank, sediment tank, biofilter, and hydroponic unit) influences the entire production process in a recirculating aquaponic system, the hydroponic component can directly affect water quality by actively removing toxic N species, and this is essential for fish growth (Yavuzcan Yildiz et al., 2017). Initially, the primary purpose of an aquaponic system was to produce fish, with vegetables being a byproduct for water treatment (Lewis, Yopp, Schramm, & Brandenburg, 1978). However, vegetables are reported to be the major revenue source when grown in tilapia-based aquaponic systems (Quagrainie, Flores, Kim, & McClain, 2018). As such, high-value vegetable crops can be considered as the primary income source where low-value fish may function as a source of fertilizer, or a byproduct.

4.3. Crops require different flow rate based on their growth rate

When optimizing flow rate in aquaponics, crop nutritional requirements and uptake ability need to be taken into consideration, a factor that has not been investigated in previous studies (Buzby et al., 2016; Diem, Konnerup, & Brix, 2017; Khater & Ali, 2015; Khater et al., 2015). Buzby et al. (2016) examined the growth performance of 34 food crops under low flow rate (18.9 L/min) and high flow rate (75.7 L/min) in an NFT aquapoonic system, and found that low flow rate reduced biomass of most plant species excepts cilantro, parsley, and minutina. Similarly, Diem et al. (2017) examined the effects of three recirculation rates (50%, 200% and 400% per day) on the growth of water spinach (*Ipomoea aquatica*), lettuce (*Lactuca sativa*), and canna (*Canna glauca*) in a deep-water culture aquaponic system and found that water spinach reduced yield under 400% recirculation rates but canna was not affected by recirculation rate especially at high fish stock density; however, it was not discussed in their study how growth rate was varied among plant species under different recirculation rates. In addition, the importance of optimizing pump size and flow rate was examined in previous studies to reduce energy demands at system-level (i.e., life cycle assessment) (Delaide et al., 2017; Love, Uhl, & Genello, 2015); however, crop growth rate was not taken into consideration in their studies. In order to determine optimal flow rate, we examined in this study the growth performance of six crop species with different growth rates. Our results suggested that HRL of 2.2 m/day was sufficient for the growth of fast-growing crops, but HRL of 3.3 m/day was necessary to maintain better growth of slow-growing crops. Fast-growing crops are known to have a greater ability to acquire nutrients, while slow-growing crops have limited capacity to acquire nutrients from solutions with low nutrient availability (Lambers & Poorter, 1992). This aspect may explain the growth variations in association with flow rate observed in our study.

Our results showed that fresh weight, dry weight, and total N concentration were higher in fast-, medium-, and slow-growing crop species in decreasing order, which indicates different nutrient demand and uptake ability among the crops associated with different growth rates. Regardless of nutrient availability, fast-growing crop species have a greater capacity to uptake nutrients and grow faster than slow-growing crop species (Lambers & Poorter, 1992). Slow-growing species are characterized by the lower specific leaf area (thinner leaves) due to relatively high concentration of cell-wall material and quantitative secondary compounds, which consume more investment (Dijkstra & Lambers, 1989; Poorter & Bergkotte, 1992), but leaf longevity is longer for slow-growing species, which could diminish nutrient losses and contribute to success in nutrient-limited

habitats (Lambers & Poorter, 1992). It is likely that there are trade-offs between growth potential and crop performance under adverse conditions.

The SPAD value was lower in fast-, medium-, and slow-growing crops in increasing order, which may be a result of tissue N dilution of fast-growing crops as nutrient concentrations can be reduced by the accumulation of new biomass, which has been discussed in previous studies (Caloin & Yu, 1984; Justes, Mary, Meynard, Machet, & Thelier-Huché, 1994; Marino et al., 2004). Therefore, careful consideration of crops is necessary when optimizing water flow rate in aquaponics as crops vary in their growth rate, nutrient removal capacity, and growth stage for harvest.

5. Conclusions

Evidence was presented in this study that water-flow rate can be optimized to potentially reduce the pumping requirement and enhance crop production in recirculating aquaponic systems. In aquaponics, compared to lower flow rate treatments, hydraulic loading rate at 3.3 m/day significantly reduced the exposure concentration and duration of detrimental level of ammonia to the roots for plants and fish. High flow rate also positively affected pH and water temperature for nitrification, ultimately increasing plant and fish production. This study implicates practical considerations that favor energy savings and higher profitability by improving crop biomass production. The flow rate optimization should consider the growth rate of plant species cultured in aquaponics. In summary, in small-scale aquaponic systems, slow-growing crops require hydraulic lauding rate as high as 3.3 m/day (6 h hydraulic retention time) to improve crop performance and yield in an aquaponics system, while fast-growing and medium-growing crops can be set up at 2.2 to 3.3 m/day (6 to 9 h hydraulic retention time).

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Table 18. Flow-rate treatments: high (HFR), medium (MFR), and low (LFR), set up for deep water culture (DWC) recirculating aquaponic systems.

Treatment	Flow rate[a] (L/min)	Flow rate (L/h)	Flow rate (L/day)	HLR[b] (m/day)	HRT[c] for DWC system (h)
HFR	2.30	138	3312	3.3	5.8
MFR	1.53	92	2203	2.2	8.7
LFR	0.77	46	1109	1.1	17.3
Recommended rate	66.7[d]	1600[d]	38400[d]	47.8[d]	0.5

[a] Flow rate of the aquaponic recirculating system.

[b] Hydraulic loading rate: Flow rate divided by total surface area of the hydroponic culture unit

[c] Hydraulic retention time: [(surface area × water depth) / flow rate]

[d] Recommended flow rates for DWC aquaponic systems used in this study. The calculations were made based on the HRT recommended by Somerville et al. (2014).

Table 19. Average values of physical and chemical water-quality parameters in fish tank for 4 weeks as affected by high (HFR), medium (MFR), or low (LFR) water flow rate.

Flow rate treatment	DO (mg/L)	Water temperature (°C)	EC (µS/cm)	pH	pH correction solution used (mL)
HFR	7.69	26.37 b	0.70 b	6.66	105.6 b
MFR	7.71	27.14 a	0.79 a	6.68	117.5 ab
LFR	7.81	27.02 a	0.76 a	6.49	134.6 a
P	ns	***	***	ns	*

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the mean of 30 replicates.

ns, *, **, *** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 20. Average concentrations of total ammonia nitrogen (TAN), nitrite (NO_2-N), nitrate (NO_3-N), phosphate (PO_4-P), and sulfate (SO_4-S) in aquaponic solution as affected by high (HFR), medium (MFR), or low (LFR) flow rate. Water samples for analyses were collected from fish tanks.

Flow rate Treatment	TAN[a]	NO_2–N[a]	NO_3–N[a]	PO_4–P[b]	SO_4–S[c]
HFR	1.19	0.45	31.9 b	43.5 b	3.56 b
MFR	1.98	0.39	38.3 a	46.4 ab	5.00 a
LFR	1.45	0.34	39.1 a	50.6 a	5.98 a
P	ns	ns	***	**	*
Correlation coefficient with flow rate	-0.13	0.03	-0.40	-0.57	-0.47
P	ns	ns	**	***	*

Means within columns followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the mean of 10 replicates. Each replicate is the mean of 10 samples collected at different dates.

ns, *, **, *** mean not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

[a] Correlation coefficient result for TAN, NO_2-N and NO_3-N were analysis from the 60 data points using HACH reaction kits.

[b] Correlation coefficient result for PO_4–P was analysis from 30 data points using ion chromatography.

[c] Correlation coefficient result for SO_4–S was analysis from the 12 data points in the last two weeks using ion chromatography.

Table 21. Plant growth parameters of fast-, medium-, and slow-growing crops grown in aquaponics for 4 weeks at high (HFR), medium (MFR), or low (LFR) flow rate.

Crop type	Flow rate	Plant height (cm)	Leaf number (n/plant)	Leaf area (cm^2/plant)	Leaf length (cm)	Specific leaf area (cm^2/g)	Leaf temperature (°C)
Fast-growing	HFR	25.3	13.8	3093.8	28.9	252.2	23.3 b
	MFR	27.1	13.5	3077.7	31.1	259.9	23.7 a
	LFR	26.9	13.8	2910.8	30.0	233.0	23.6 a
	ns	ns	Ns	ns	ns	ns	**
Medium-growing	HFR	40.6	28.2	1647.4	36.5	120.5	23.6 b
	MFR	39.0	35.3	1554.2	33.5	119.3	24.0 a
	LFR	35.1	33.1	1515.5	30.7	112.1	24.1 a
	ns	ns	Ns	ns	ns	ns	**
Slow-growing	HFR	36.3	36.9	1403.3	25.1	117.5	23.8 b
	MFR	34.5	34.2	1306.9	21.6	116.3	23.8 b
	LFR	34.4	34.4	1193.6	20.5	113.7	24.2 a
	ns	ns	Ns	ns	ns	ns	**
ANOVA							
Crop type		***	***	***	***	***	***
Flow rate		ns	ns	ns	ns	ns	***
Crop type × Flow rate		ns	ns	ns	ns	ns	*

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively. Each crop species consisted of 12 replicates.

ns,*,**,*** mean no significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 22. Fresh and dry weights of fast-growing, medium-growing, and slow-growing crops grown in aquaponics with low flow rate (LFR), mediate flow rate (MFR), and high flow rate (HFR).

Crop type	Flow rate	Fresh weight (g/plant)			Dry weight (g/plant)		
		Total	Shoots	Roots	Total	Shoots	Roots
Fast-growing	HFR	323.0 a	287.8 a	35.2 a	9.6 a	8.9 a	0.67
	MFR	332.3 a	300.1 a	32.2 a	9.9 a	9.2 a	0.66
	LFR	278.4 b	249.6 b	28.8 b	7.1 b	6.4 b	0.71
		**	**	*	**	**	ns
Medium-growing	HFR	198.3 a	153.0 a	45.0	10.5 a	9.4 a	1.09
	MFR	157.6 ab	126.7 ab	29.8	8.7 ab	7.6 ab	1.11
	LFR	144.4 b	101.6 b	32.3	7.8 b	6.8 b	0.96
		*	*	ns	*	*	ns
Slow-growing	HFR	154.7 a	115.6	39.1 a	9.3 a	8.3 a	0.95
	MFR	147.9 b	112.4	35.5 ab	7.1 b	6.0 ab	1.06
	LFR	123.4 b	91.5	31.9 b	6.8 b	5.8 b	1.01
		*	ns	*	*	*	ns
ANOVA							
Crop type		***	***	***	***	***	***
Flow rate		***	**	***	**	**	ns
Crop type × Flow rate		*	*	ns	ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively. Each crop species consisted of 12 replicates.
ns,*,**,*** mean no significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Table 23. Fish production in aquaponics grown for 4 weeks at high (HFR), medium (MFR), or low (LFR) flow rate.

Flow rate	Fish feed applied (g)	Water replenishment (L)	Initial stocking density (kg/m³)	Final stocking density (kg/m³)	Fish biomass gain[a] (kg/tank)	SGR[b] (%)	FCR[c]
HFR	1980	203	19.5	26.5	2.7 a	9.5 a	0.69
MFR	1980	177	19.3	24.4	1.9 ab	6.6 ab	1.06
LFR	1980	133	19.3	22.4	1.18 b	4.2 b	1.55
P	–	ns	ns	*	*	*	–

[a] Treatment with the different number is significant at the p = 0.05 level.

[b] Specific growth rate calculated as SGR = (ln final weight of fish−ln initial weight of fish) × 100/days.

[c] Feed conversion ratio calculated as FCR = total weight of fish feed applied/total fish biomass increase (wet weight).

Table 24. Total nitrogen content (g/plant) and SPAD value of fast-growing, medium-growing, and slow-growing crops grown in aquaponics at low flow rate (LFR), mediate flow rate (MFR), or high flow rate (HFR).

Crop type	Flow rate	Total nitrogen (g/plant)			SPAD
		Total	Shoots	Roots	
Fast-growing	HFR	0.65 a	0.61 a	0.09 a	19.4 a
	MFR	0.58 b	0.51 b	0.08 b	19.1 a
	LFR	0.31 c	0.27 c	0.07 c	14.5 b
		*	*	*	**
Medium-growing	HFR	0.47 a	0.43 a	0.05 a	32.6 a
	MFR	0.34 ab	0.35 ab	0.02 b	28.1 b
	LFR	0.28 b	0.27 b	0.02 b	22.4 c
		*	*	*	***
Slow-growing	HFR	0.38 a	0.34 a	0.04 a	30.4 a
	MFR	0.27 ab	0.25 ab	0.02 b	29.7 ab
	LFR	0.20 b	0.18 b	0.02 b	27.7 b
		*	*	*	*
ANOVA					
Crop type		**	***	*	***
Flow rate		*	*	*	***
Crop type × Flow rate		ns	ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively. Each crop species consisted of 3 replicates.

ns,*,**,*** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 25. Plant photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration, and intrinsic water use efficiency (WUE) of crops grown in aquaponics with high- (HFR), medium- (MFR), or low- (LFR) flow rate. The values are photosynthetic parameters measured at day 21 after transplanting.

Crop type	Flow rate	Photosynthetic rate (Pn) (μmol CO_2/m^2/s)	Stomatal conductance (gs) (mol H_2O/m^2/s)	Transpiration rate (E) (mmol H_2O/m^2/s)	Intercellular CO_2 concentration (Ci) (μmol CO_2 /m^2/s)	WUE (μmol CO_2 mmol/ H_2O)
Fast-growing	HFR	12.87 a	0.37	8.02	303.02 b	1.68
	MFR	11.96 ab	0.39	7.17	314.65 a	1.71
	LFR	9.25 b	0.33	6.83	317.72 a	1.41
		**	ns	ns	*	ns
Medium-growing	HFR	13.38 a	0.52 a	8.74 a	320.29	1.55
	MFR	11.65 ab	0.43 ab	7.05 b	320.83	1.79
	LFR	10.06 b	0.39 b	6.60 b	324.23	1.59
		*	*	*	ns	ns
Slow-growing	HFR	14.39 a	0.40 a	8.45 a	298.55	1.86
	MFR	12.54 ab	0.31 b	5.94 b	295.57	2.27
	LFR	11.50 b	0.29 b	5.84 b	299.31	2.19
		*	**	***	ns	ns
ANOVA						
Crop type		ns	***	ns	***	***
Flow rate		***	**	***	ns	ns
Crop type $\times$ Flow rate		ns	ns	ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively. Each crop species consisted of 12 replicates.

ns,*,**,*** mean no significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Figure 10. Schematic diagram of each experimental aquaponics unit.
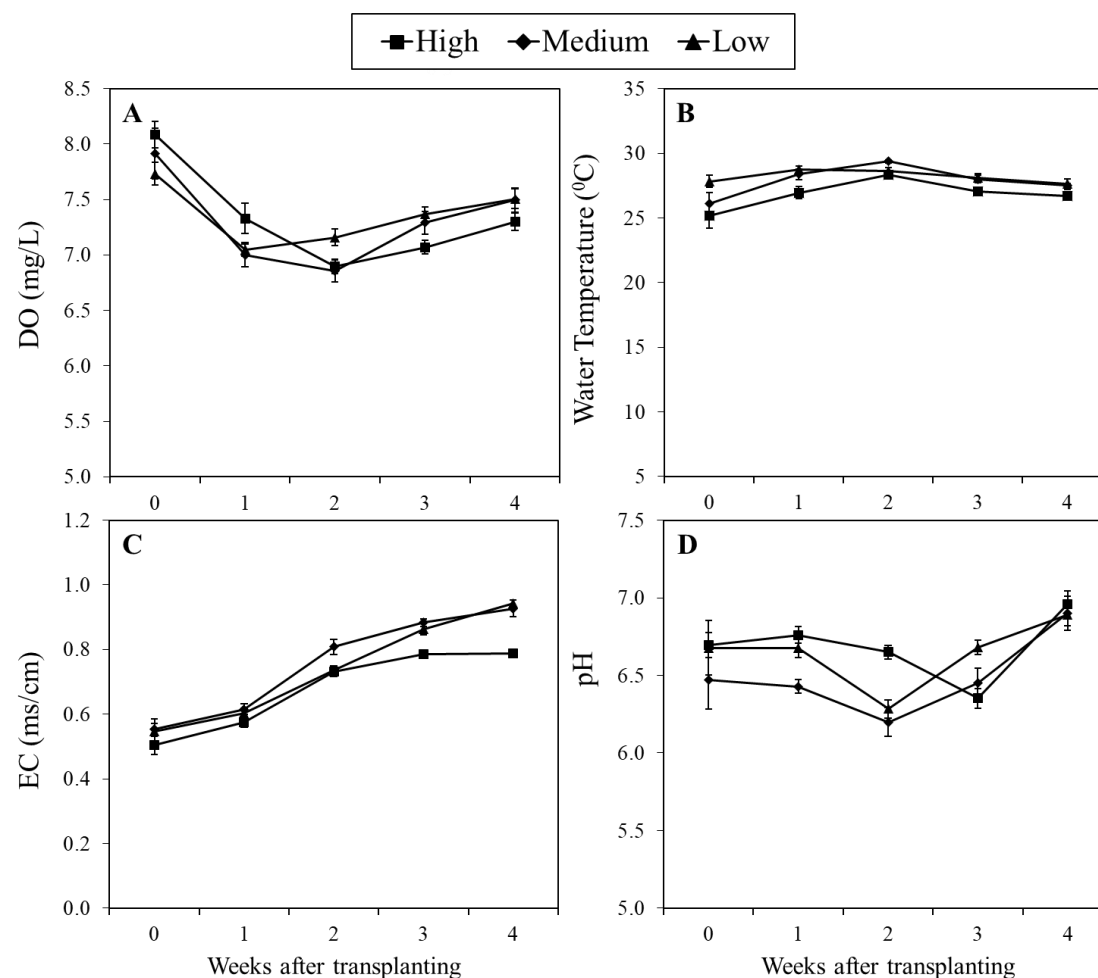
Figure 11. Weekly changes in (A) dissolved oxygen (DO), (B) water temperature, (C) electricity conductivity, and (D) pH as affected by flow rate treatments: high (HFR), medium (MFR), and low (LFR) flow rate. Water samples were measured daily from fish tank and grow bed in the aquaponic system. Average concentrations of temperature and electricity conductivity in HFR, MFR, and LFR were given for every week. Each data point is the mean of 7 replicates ± SE.
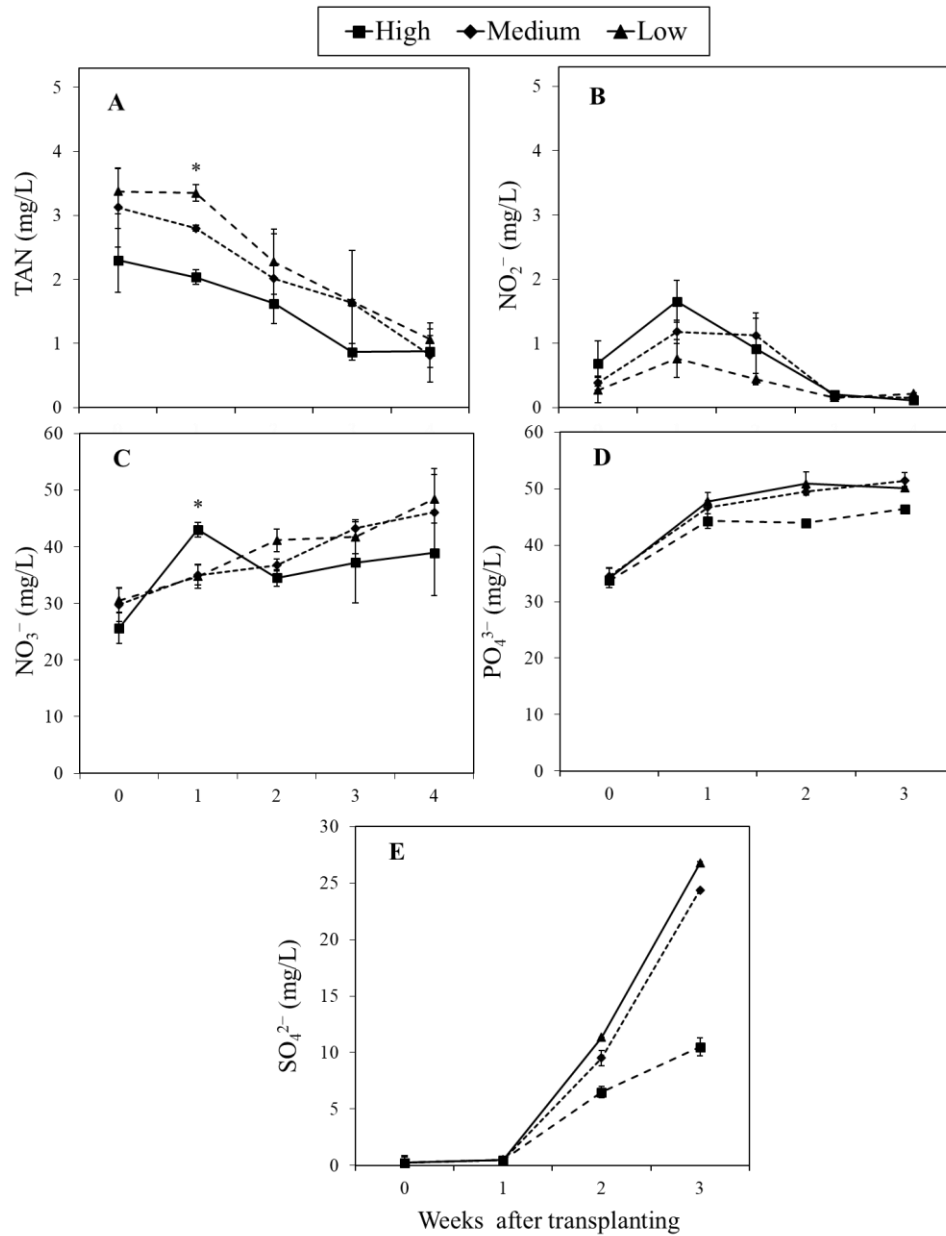
Figure 12. (A–E) Dynamic changes in nitrogen species, A = total ammonium nitrogen (TAN), B = nitrite (NO_2^-), C = nitrate (NO_3^-), D=phosphate (PO_4^{3-}) and E=sulphate (SO_4^{2-}) over 4 weeks as affected by flow-rate treatments. Data were measured every three days and weekly data were combined to show significant differences. Each data point is the mean of 4 replicates ± SE.
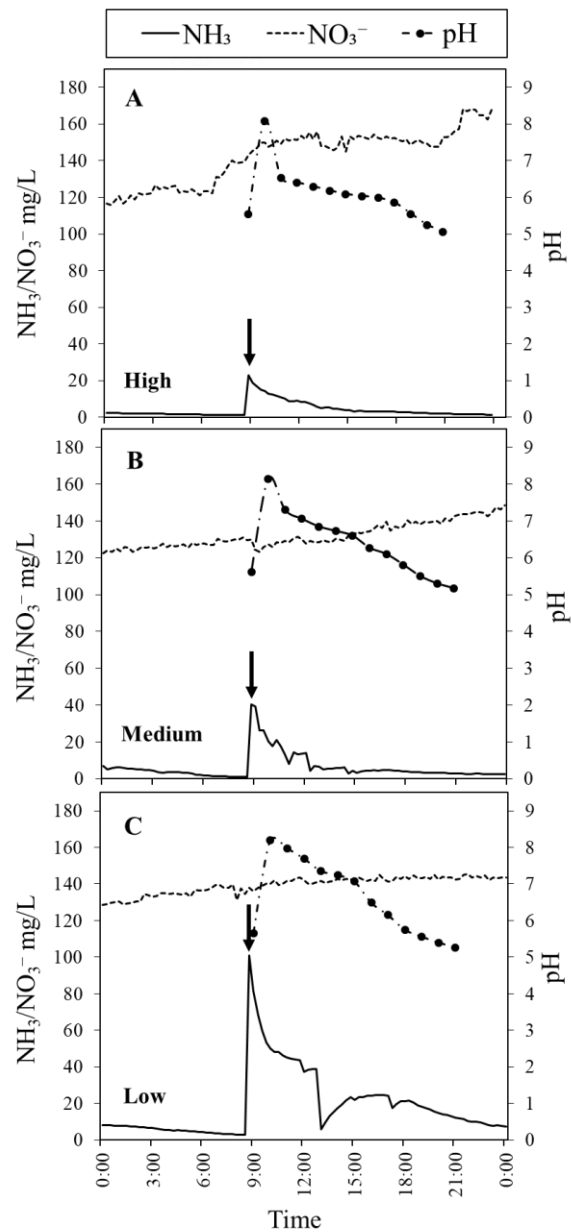
Figure 13. (A–C) Dynamic changes in ammonia (NH_3), nitrate (NO_3^-), and pH over 24 hours as affected by flow-rate (high, medium, and low) treatments, which were measured in the absence of plants. Arrow indicates the time when fish feed (0.5% fish fresh weight) was applied to the fish tank.
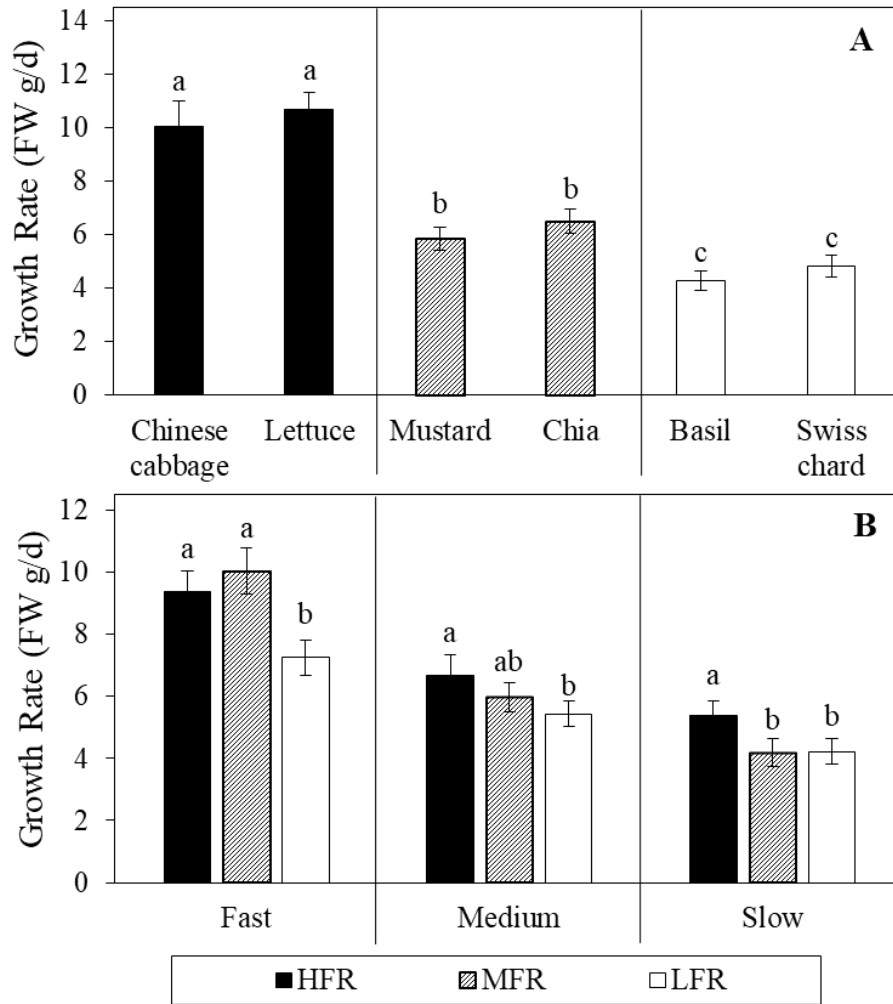
ANOVA	
Crop type	***
Flow rate	**
Crop type × Flow rate	ns

Figure 14. (A) Daily growth rate of six crop species with fast- (Chinese cabbage, lettuce), medium- (mustard, chia), and slow- (basil, Swiss chard) growth rate in aquaponics and (B) their growth rate as affected by high (HFR), medium (MFR), or low (LFR) flow rate. The same letter within the same plant category is not significantly different based on Tukey's honestly significant difference test (α =0.05). Data represent mean values ± SE (n = 6).
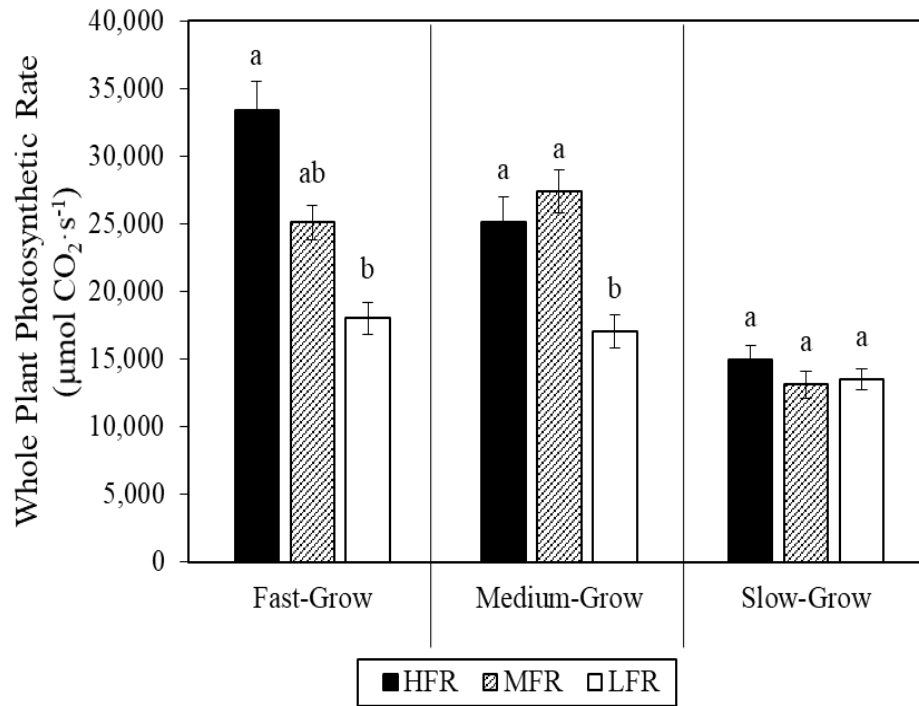
ANOVA	
Crop type	***
Flow rate	***
Crop type × Flow rate	ns

Figure 15. Whole plant photosynthetic rate of fast-, medium-, and slow-growing crops in aquaponics as affected by high (HFR), medium (MFR), and low (LFR) flow rate. The same letter within the same plant category is not significantly different based on Tukey's honestly significant difference test (α =0.05). Data represent mean values ± SE (n = 6).

Table S4. Plant-growth parameters of fast-growing, medium-growing, and slow-growing crops measured at Day 0 before transplanting.

Crop type	Flow rate	Plant height (cm)	Leaf number (n/plant)	Total fresh weight (g/plant)	SPAD	Leaf length (cm)
Fast-growing	High	7.27	4.5	17.11	23.29	11.61
	Medium	6.80	4.4	17.06	22.52	12.37
	Low	6.98	4.6	16.96	23.03	11.81
		ns	ns	ns	ns	ns
Medium-growing	High	12.63	3.5	17.57	30.62	17.27
	Medium	12.17	3.2	17.57	29.35	15.68
	Low	10.98	3.5	17.53	28.77	14.37
		ns	ns	ns	ns	ns
Slow-growing	High	8.14	5.5	17.74	32.63	6.87
	Medium	10.73	8.1	19.26	30.45	7.41
	Low	8.79	6.1	18.23	30.81	6.75
		ns	ns	ns	ns	ns
Significance						
Crop type		***	***	***	***	***
Flow rate		ns	ns	ns	ns	ns
Crop type × Flow rate		ns	ns	ns	ns	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively. Each crop species consisted of 12 replicates.

ns,*,**,*** mean no significant or significant at P≤0.05, 0.01, or 0.001, respectively.

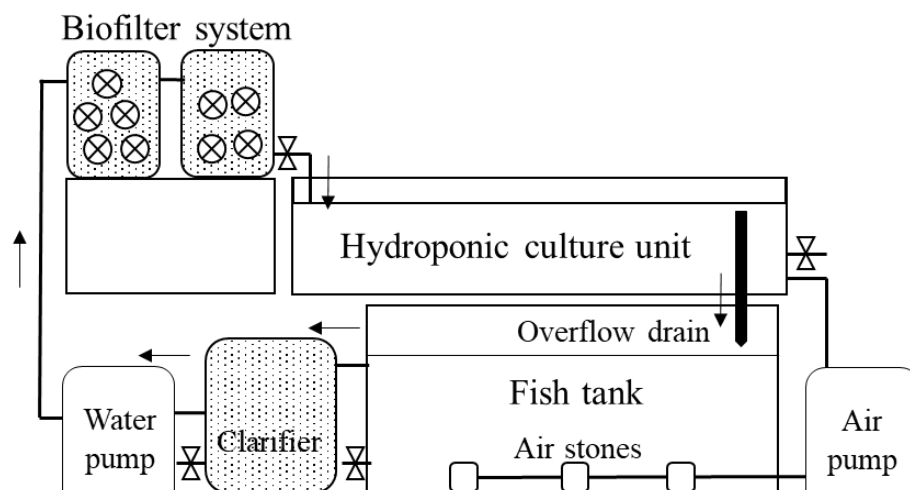**Figure S3.** Fresh weight of different crop species grown in aquaponics with high flow rate, mediate flow rate, and low flow rate.

COMPARISONS OF NITROGEN AND PHOSPHORUS MASS BALANCE FOR LETTUCE-, BASIL-, AND TOMATO-BASED AQUAPONICS AND HYDROPONICS

Abstract

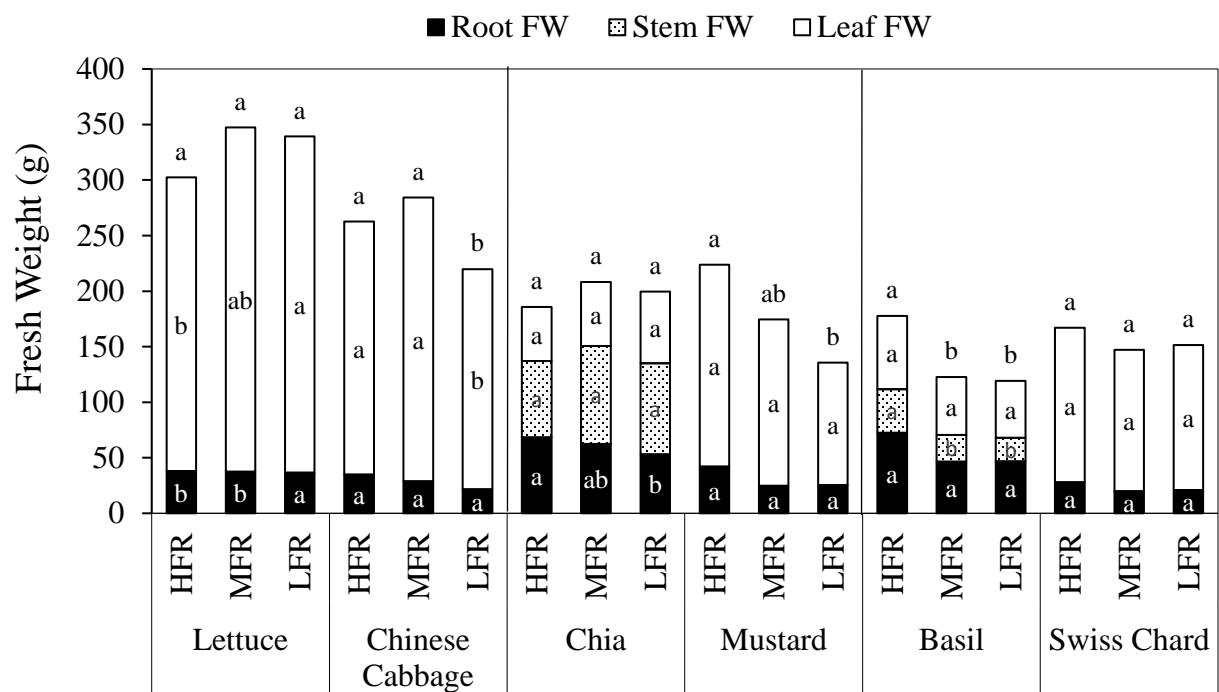Nitrogen (N) and phosphorus (P) are important macronutrients for the production of fish and plant crops in aquaponic systems, but also significant sources of environmental contamination. Although aquaponics is known to be the most efficient agriculture production system by far, it has yet to be optimized due to the limited information on N and P mass balances. This study compared aquaponic systems with hydroponic systems to assess the N and P distributions, N and P use efficiency (NUE and PUE), and potential environmental impacts. Cherry tomato, basil, and lettuce were cultured in recirculating tilapia (*Oreochromis niloticus*)-based aquaponic or stand-alone hydroponic systems over a 3-month period. N and P mass balances were developed by using N and P concentrations in fish feed, solid waste, wastewater, fish biomass, and plant biomass for aquaponic systems, and chemical fertilizer, wastewater, and plant biomass for hydroponic systems, which were to estimate N and P losses from each system via denitrification and P precipitations, respectively. Estimated total N loss ranged from 59.2 to 69.5% from aquaponics via solid waste, wastewater, and denitrification, while it ranged from 75.7 to 86.5% from hydroponics via wastewater and denitrification. Similarly, the estimated total P loss ranged from 38.4 to 53.6% from aquaponics via solid waste, wastewater, and P precipitations, while it ranged from 78.6 to 89.1% from hydroponics via wastewater and P precipitations. Crop species had a significant impact on N and P distribution, and tomato was most effective in reducing N loss and increasing N allocation to plant parts followed by basil and lettuce. Tomato-based aquaponics had better water quality (lower total NH_3–N and NO_2–N), nutrient removal rate, fish growth (lower feed conversion ratio or FCR), and plant yield than those of basil or lettuce-based aquaponics. When edible plant parts were taken into consideration, NUE was 0 to 38.5% lower for aquaponics than for hydroponics. However, when both fish and plant crop biomass were considered, the NUE of aquaponics was 70.8 to 114.3% higher than for hydroponics. Meanwhile, PUE was higher for aquaponics than for hydroponics even only when edible parts were considered and when the biomass of both crops were considered, PUE for aquaponics was 335.7 to 369.2% higher than for hydroponics. Importantly, our mass balance analysis indicated that hydroponic systems had 1.2-1.6 and 2.5-4.2 times higher N and P losses, respectively, than did aquaponics, which was mainly due to 5-9 and 5-7 times higher concentrations of N and P inputs, respectively, causing higher N gas emission and organic P precipitations. Our N and P mass-balance analyses suggested that reducing nutrient inputs is critical to improve N and P use efficiency for both aquaponics and hydroponics, which should be combined with proper crop choice, operation conditions, and management practices to further improve the efficiency of the systems.

1. Introduction

Rapid population growth and urbanization in the 21st century raise the demand for food production with less water and energy consumption. Since urban areas lack soil due to both built infrastructure and soil contamination, there is strong need to introduce sustainable, soilless crop-production systems in urban areas. As one of the most widely used soilless production systems, hydroponics utilizes nutrient solutions with or without soilless substrate rather than soils *per se* to culture crops (Kim, Yang, Lin, & Langenhoven, 2018). Although hydroponics could yield up to 11 times more per unit area than conventional soil-based agriculture (Barbosa et al., 2015; Rouphael et al., 2004), it relies on luxuriant fertilizers including N and P, and, therefore, requires an alternative source of fertilizer to synthetic fertilizer to increase its sustainability (Lommen, 2007; Molitor, 1990). Replacement of fertilizer with aquaculture wastewater is one way to achieve this with the extra benefits of obtaining fish protein using an integrated food production within a closed loop, which is also called "aquaponics" (Rakocy, 1993).

Aquaponics is a recycling system combining aquaculture and hydroponics in which the wastewater from the aquaculture subsystem is reused by culturing crops in the hydroponic subsystem. Fish release ammonia nitrogen (NH_3–N) via urine by transamination and deamination of feed protein (Wongkiew et al., 2017). Nitrifying bacteria convert ammonium (NH_4–N) into nitrate (NO_3–N) by nitrification with nitrite (NO_2–N) as the intermediate product, then surplus nutrients including NH_4–N and NO_3–N dissolved in the wastewater are absorbed by plant crops, and then cleaner water flows back to the aquaculture sub-system in the fish tank. In aquaponic systems, at least two cash crops (e.g., fish and plant crops) can be produced by reusing aquaculture wastewater (Diver, 2006; Rakocy, Masser, & Losordo, 2012; Tyson, Treadwell, & Simonne, 2011). While the benefits of fish and plant co-cultivation were realized more than 1,500 years ago (Coche, 1967), this integrated system has remained extremely limited due to a lack of quantitative research to support the development of economically feasible aquaponic systems (Goddek et al., 2015). In most commercial aquaculture systems, daily discharge of 10–40% wastewater during and at the end of fish cultivation is commonly practiced as a result of the accumulation of NH_3–N and soluble organic matter (Klinger & Naylor, 2012). Even in aquaponic studies, up to 20% of wastewater was discharged to avoid inorganic N species accumulation and to maintain good water quality (**Table 32**). Thus, designing a 100% recirculating, zero-discharge aquaponic system is essential for environmental and economic sustainability.

N and P are macronutrients and key nutrients for crop production. A large number of aquaculture studies showed that less than 30% of N contained in fish feed is assimilated through fish harvest (Avnimelech, 1999; Brune et al., 2003; Piedrahita, 2003; Siddiqui & Al-Harbi, 1999), and the remaining N is dissolved into the surroundings as the NH_3–N-rich aquaculture effluent and processed via nitrification under aerobic conditions. In an aquaponics system, microbes are used as a biofilter to convert NH_4–N into NO_3–N that is assimilated into plant biomass, and NH_3–N and NO_2–N should be kept at low levels to reduce the potential for fish and plant toxicity (Oke, 1966; Rakocy, 1993). The fate of N species in aquaponics can be expected to vary with fish and plant species, production-system design, feed formula, and nutrient management (Smith et al., 2002),

and therefore a sensitive and efficient indicator is needed to compare and assess the performance of aquaponic production systems.

N-use efficiency (NUE) is often used as an indicator to demonstrate the efficiency of an agricultural production system (Harrington, Fownes, & Vitousek, 2001; Morell, Lampurlanés, Álvaro-Fuentes, & Cantero-Martínez, 2011; Peng et al., 1996; Stamatiadis, Tsadilas, Samaras, Schepers, & Eskridge, 2016; Yang & Kim, 2019; Zhang, Wang, Gong, Xu, & Mo, 2019). As an integrated production system, NUE can be calculated in aquaponics through the ratio of total N input, mostly provided by fish feed and total N recovered via biomass production of crops. Released from fish digestion, aquaculture wastewater is rich in dissolved and solid wastes primarily containing N, P, and uneaten feed (Summerfelt & Clayton, 2003). To increase NUE and PUE, most N and P derived from fish feed should be directly used for crop biomass production in aquaponic systems and less should be wasted. There is limited information on NUE in aquaponic systems, and virtually no information is available on PUE. Further, while aquaponic systems are known to be efficient compared to hydroponic systems, there are no quantitative data to support this premise. Compared to traditional hydroponic production, aquaponics is characterized by lower nutrient availability (Yang & Kim, 2019), higher pH (slightly higher than 7) to satisfy the pH requirement for fish (7 to 9), plants (5.5 to 6.5), and nitrifying bacteria (7 to 9) at the same time, and lack of essential nutrient elements required for plant crops. The nutrient formula of fish feed is significantly different from that of hydroponic-luxuriant fertilizer, which is deficient in potassium, calcium, magnesium, and iron (Chapter 2, this dissertation; Seawright, Stickney, & Walker, 1998). However, luxuriant fertilizer in hydroponics has been reported to lead to serious imbalances in nutrient uptake and reduces nutrient-use efficiency (Oscar & Aline, 2016; Raviv & Lieth, 2007; Sabli, 2012; Yang & Kim, 2019). Therefore, nutrient mass balance and nutrient-use-efficiency research needs to be done to evaluate the efficiency of aquaponic and hydroponic systems and compare the growth and productivity of plants grown in these systems.

To date, most studies have focused only on the N budget (Delaide et al., 2017; Endut, Jusoh, & Ali, 2014; Fang et al., 2017; Wongkiew et al., 2017; Zou et al., 2017), and the P budget in aquaponics systems is largely unexplored (B. da S. Cerozi & Fitzsimmons, 2017; Delaide et al., 2017; Jaeger et al., 2019). Further, little consideration has been made regarding effects of plant crops on N budget, while virtually no information is available on effects of plant crops on P budget. Only a few studies compared the aquaponic and hydroponic systems (Pantanella et al., 2012; Roosta & Afsharipoor, 2012; Suhl et al., 2016), but mainly focused on comparing crop performance and yield, not efficiency of the systems.

The objective of this study was to evaluate the efficiency of aquaponic systems by performing a mass-balance approach. We also characterized N and P use efficiency and water efficiency in fully recirculating aquaponic systems using three different crops, cherry tomato, basil, and lettuce, that are distinctive of their growth and yield characteristics. These evaluations are important to provide critical information for the achievement and optimization of sustainable zero-discharge aquaponic production system.

2. Materials and methods

2.1. Experimental setup and operation

Experiments were conducted in the greenhouse at Purdue University in West Lafayette, IN (lat. 40°N, long. 86°W). Three aquaponics and three hydroponics systems were used in the study. Each aquaponic system consisted of a 350-L fish tank, a two-stage biofilter (up-flow and down-flow, total volume = 40 L) (Wongkiew, Popp, et al., 2017) filled with 1.5 kg of bio-filter media (Kaldnes K1 Media, Aquatic Eco-System, Apopka, FL, USA), and a 350-L, 1-m^2 hydroponic culture unit utilizing a Styrofoam foam board (**Figure 16 A**). A peristaltic water pump was used to deliver water from the fish tank to the hydroponic unit. Aeration in the fish tank and in the hydroponic unit was provided by an air pump (Super Pond, Liner Air Pump, Kennewick, WA). Each fish tank was stocked with 20 kg/m^3 Nile tilapia (*Oreochromis niloticus* L.) fish, which were obtained from the Animal Sciences Research and Education Center at Purdue University and had been cultivated in a conventional aquaculture system for 4 months prior to this study. At the beginning and the end of the study, fish mass in each aquaponic system was measured using an analytical balance and fish number was counted. Fish were fed at 9:00 am once daily at an average rate of 1% of body weight with commercial fish feed, which was composed of 41% protein and 1.1% P (Purina® AquaMax® Sport Fish MVP, USA), with a particle size of 4.8 mm (**Table 26**). Each hydroponic unit and fish tank had air stones to maintain dissolved oxygen (DO) concentrations at or above 5 mg/L. The fish tank was covered with a plastic board to prevent algal growth. There was a lid (0.5 m × 0.5 m) on each board which could be open to permit light into the fish tank during daytime. An aquarium thermostat heater (Eheim Jager TruTemp, Germany) was used to maintain water temperature of the fish tank within the optimum range of 26–28°C for tilapia culture in aquaponic systems. Similarly, each hydroponic system consisted of a 350-L nutrient solution reservoir and a 350 L or 1 m^2 deep water culture (DWC) hydroponic unit (**Figure 16 B**). The recirculating hydroponic system was used as a control for comparisons with the aquaponic system. A commercial plant nutrient solution (**Table 26**) was used as an initial and replenished nutrition solution to readjust electrical conductivity (EC) at 2.0 mS/cm periodically. Before the study, reverse osmosis water was used to fill up both aquaponic and hydroponic systems and then to replenish transpiration and evaporation losses during the study.

2.2. Plant materials and cultural methods

Nile tilapia (*Oreochromis niloticus*) were obtained from Animal Sciences Research and Education Center at Purdue University, which had been cultivated in a conventional aquaculture system for 4 months prior to the start of the experiment. Three different plant species, cherry tomato (*Lycopersicon esculentum* 'Washington Cherry'), a fruity crop; basil (*Ocimum basilicum* 'Genovese'), an herb crop; and lettuce (*Lactuca sativa* 'Cherokee'), a leafy crop, were cultured in each aquaponic and hydroponic system. These crops were chosen for their popularity in aquaponic and hydroponic production systems while displaying different growth and harvest characteristics. Due to the differences in production duration (90 days for cherry tomato and basil; 30 days for lettuce), the study was divided into three phases based on the production period of lettuce: the first production period (30 days; Phase I), the second production period (30-60 days; Phase II),

and the last production period (60-90 days; Phase III) (**Figure 18**). Seeds were purchased from a commercial source (Johnny's Selected Seeds, Winslow, ME) and sown in Agrifoam (Syndicate Sales, Inc., Kokomo, IN) trays with a few days intervals, and seedlings with similar size were selected at the time of transplanting. Seeds were initially irrigated with tap water, followed by a half-strength fertilizer solution once germinated, and full-strength fertilizer after seedlings developed true leaves (Kim et al., 2018). The fertilizer was a combination of two water-soluble fertilizers (3:1 mixture of 15N–2.2P–12.5K Cal-Mag Special and 21N–2.2P–16.6K Multi-Purpose fertilizers, respectively; Everris NA, Dublin, OH). The fertilizer consisted of (mg/L): 150 nitrogen (N), 20 phosphorous (P), 122 potassium (K), 38 calcium (Ca), 15 magnesium (Mg), 0.8 iron (Fe), 0.4 manganese (Mn) and zinc (Zn), 0.2 copper (Cu) and boron (B), and 0.1 molybdenum (Mo). Nitrate form was 76% of N provided. After the third true leaf of seedlings appeared, uniform healthy seedlings were chosen and transplanted into mesh pots containing 85 g clay pebbles, then transferred to hydroponic units of aquaponics and hydroponics. The planting density was 8 plants/m^2 for cherry tomato and 24 plants/m^2 for basil and lettuce per aquaponic unit. A mixture of potassium hydroxide (2N) and saturated (0.05N) calcium hydroxide (v:v=1:1) was directly added to the fish tank in aquaponics to adjust pH at to 7 daily before feeding, while pH in hydroponics was adjusted to 5.5 to 6.0 by directly adding adjustment solution to the nutrient solution reservoir. Dissolved oxygen (DO) concentrations, temperature, EC, and pH were measured daily *in situ* using the HQ40d Portable Water Quality Lab Package (HACH Corp., Loveland, CO, USA). Daily fish feed consumption was recorded.

Photoperiod was 14 h (8:00 am to 10:00 pm) consisting of natural daylight with supplemental lighting using high-pressure sodium (HPS) lamps. Day (7:00 am to 9:00 pm) and night (9:00 pm to 7:00 am) temperatures were set at 25 and 22°C, respectively. A supplemental photosynthetic photon flux (PPF) was measured using a quantum sensor (LI-250A light meter; LI-COR Biosciences, Lincoln, NE). The production temperature, daily light integral, and vapor pressure deficit (VPD) were recorded (**Figure S4**), which were averaged at 23.6 °C, 8.7 mol/m^2/d, 0.73 kPa, respectively.

2.3. Water sampling and nutrient analytical methods

Water samples were obtained from both fish tank and hydroponic units every 4 days before feeding at 9:00 am and were analyzed immediately for total ammonia nitrogen (TAN), nitrite (NO$_2$–N), nitrate (NO$_3$–N), and phosphate (PO$_4$–P) concentrations, using HACH reaction kits (Loveland, Co. Ltd., USA), namely Ammonia Reagent Powder Pillows, Nitrite Reagent Powder Pillows, Nitrate Reagent Powder Pillows, and Phosphate Reagent Powder Pillows, respectively.

2.4. Sampling and fresh and dry mass measurements

All plant samples were weighed to get an average fresh mass at the beginning, during, and at the end of the study. Plant-biomass increment was calculated as the mass difference between the beginning and during (week 8 for tomato and basil; week 3 for lettuce) the study.

At the end of experiment, plant tissues (roots, leaves, and/or stems) were separated and immediately weighed using an analytical balance for the determination of fresh mass. Dry mass

was obtained by drying each sample in a forced-air oven at 70°C (Heratherm OMH400, Thermo Scientific Inc., Waltham, MA, USA) for 5 days until constant mass was reached. Tomato fruit fresh and dry mass were recorded weekly during harvest season (week 9 to week 12) and used for the calculation of total fresh and dry mass of fruit. Fruit dry mass was obtained by placing each fruit on a metal tray and transferring to a drying oven for 1 h at 100 °C, followed by 65 °C until fruit was completely dried.

At the beginning and end of an experiment, 5 representative fish samples were randomly collected from each aquaponics system and weighted to get a five-fish mass, then an individual fish mass was calculated by dividing the five-fish mass by the number of fish weighed; this procedure was repeated five times to obtain an average individual fish mass for each aquaponic system unit. The fish stocking density was calculated as the total fish mass (average individual fish mass multiplied by fish number) in each aquaponic system at the beginning of the study divided by the volume of the fish tank (350 L). The fish biomass increment was calculated by difference of total fish mass in each aquaponics system at the beginning and end of an experiment. The feed-conversion ratio (FCR) was calculated from the relationship of feed intake and mass gain, by the following formula: $FCR = m_c/(m_f-m_i)$, where m_f=final mass, m_i=initial mass and m_c=amount of feed applied. Then the fish meat, inner organs, and bones were separated and immediately weighed for the determination of fresh mass. Dry mass was obtained by drying each sample in a forced-air oven at 70°C for 5 days until constant mass was reached.

Solid waste was collected from the clarifier tank where most solid waste was found. Excess water was carefully drained, then the solid waste was immediately transferred to a drying oven set at 70°C in a stainless-steel pan and dried completely until a constant mass was reached.

2.5. Total N and P measurements

At the end of the experiment, five representative fish samples were collected from each aquaponic system. Harvested fish were bled, gutted, and cut into fillet. Dry mass of fish fillets was obtained by drying each sample in a forced-air oven at 70°C for 5 days until constant mass was reached. All dried samples of plants, fish fillets, solid waste, and fish feed were ground through a 10-mesh sieve with a Wiley mini mill (Thomas Scientific, Swedesboro, NJ, USA) for total N and total P content analyses. When preparing samples for total N analysis, a clean small sampling spatula was used to place 30.0 mg of sample material into an empty sample tin, which was then carefully wrapped into a ball. The total N content of sample balls was measured using the FlashEA (C/N machine, Swedesboro, NJ, USA) and determined as described by Bhattacharyya et al. (2015).

When preparing samples for total P analysis, each sample was weighted into 0.07 g aliquots, and the sample was transferred into a 20 mL glass vial, then ashed in a muffle furnace at 495°C for 8 hours. Then each sample was mixed with 8 ml of 100 mN hydrochloric acid (100 mN HCl) and 1.6 mL Reagent B (50 ml of Reagent A + 12 ml distilled water + 0.264 g L-ascorbic acid; 2 L Reagent A: 12 g ammonium molybdate + 0.2908 g antimony potassium tartrate + 1 L 5 N sulfuric acid), then wait 30 minutes for full color development. Samples were diluted 40-fold by adding Millipore-filtered water and added with a reagent liquid following the protocol of a colorimetric method for phosphorus analysis (Murphy & Riley, 1962). The total P content of each sample was

analyzed by Epoch microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) with a wavelength of 880 nm.

2.6. N and P use efficiency

In aquaponics, the ratio of N and P assimilated by fish and plant crops for biomass production resulting from total N and P input from fish feed was defined as N-use efficiency (NUE) and P-use efficiency (PUE), respectively.

NUE in aquaponics was calculated as below:

$$\text{NUE}=\frac{N_{plant}+N_{fish}}{f_N \cdot M_f \cdot T} \tag{1}$$

NUE in hydroponics was calculated as below:

$$\text{NUE}=\frac{N_{plant}}{C_N \cdot M_c \cdot T} \tag{2}$$

PUE in aquaponics was calculated as below:

$$\text{PUE}=\frac{P_{plant}+P_{fish}}{f_P \cdot M_f \cdot T} \tag{3}$$

PUE in hydroponics was calculated as below:

$$\text{PUE}=\frac{P_{plant}}{C_P \cdot M_c \cdot T} \tag{4}$$

Where, f_N and f_P are the fractions of N and P in fish feed (g N/g; g P/g), respectively; C_N and C_P are the fractions of N and P in chemical fertilizer solutions (g N/L; g P/L), respectively; M_f is the feeding rate (g/day); T is the production duration (days); M_c is the chemical fertilizer application rate (L/day); N_{plant} and P_{plant} are the average N and P assimilated by plant crops at harvest (g N; g P), respectively; N_{fish} and P_{fish} are the average N and P assimilated in fish meat, inner organs, scales, and bones (g N; g P), respectively.

2.7. Estimating N and P mass balance

Mass balance was completed for aqueous phase N and P using the data collected in this study, literature values, and calculated values. **Figure 17** conceptualizes the mass balance completed for N and P derived from fish feed. Since reverse osmosis (RO) water contained a negligible amount of nutrient elements (Chapter 2, this dissertation), a nutrient contribution from RO water was not considered in this study. Therefore, the only source of nutrients in this zero-discharge aquaponic system was from fish feed added daily, and the only source of nutrients in this zero-discharge hydroponics system was from fertilizer solution added daily. In order to determine the mass of aqueous N and P in the aquaponic system, the mass of aqueous N species (TAN, NO_2–N, NO_3–N) and P (PO_4–P) entering the hydroponic culture unit were measured weekly. In order to determine N and P content in fish, The actual total N and P concentrations measured from dried fish meat were used to estimate the total N and P in fish inner organs, scales, and bones using literature

values: a protein content of 42%, the conventional 6.25 nitrogen-to-protein conversion ratio (Boxman et al., 2018; Piedrahita, 2003), and the P of 45% (Piedrahita, 2003), respectively. Total N and P contents in solid waste were determined by multiplying total g dry mass solid waste by g N and g P concentrations in a measured sample of g dry mass solid waste. The total N and P contents of plant crops were calculated by summing up the total N and P contents (total N and P concentration per g tissue multiplied by tissue dry biomass) in all plant-organ tissues (leaf, stem, root, and fruit, if applicable). At the end of the study, the mass of N species and P retained in the aquaponic and hydroponic systems were determined, and total N and P in the aqueous phases of aquaponic and hydroponic systems were also measured.

The mass balances of N or P in aquaponic and hydroponic systems during the study period were calculated as below (Wongkiew et al., 2017):

The mass balance of N in aquaponics:

$$f_N \cdot M_f = \frac{d}{dt}(C_{TAN} + C_{NO2-N} + C_{NO3-N})V + \frac{N_{plant}}{T} + \frac{N_{fish}}{T} + \frac{N_{sed}}{T} + \frac{N_{loss}}{T} \tag{5}$$

The mass balance of N in hydroponics:

$$C_N \cdot M_c = \frac{d}{dt}(C_{TAN} + C_{NO2-N} + C_{NO3-N})V + \frac{N_{plant}}{T} + \frac{N_{loss}}{T} \tag{6}$$

The mass balance of P in aquaponics:

$$f_P \cdot M_f = \frac{d}{dt}(C_{PO4-P})V + \frac{P_{plant}}{T} + \frac{P_{fish}}{T} + \frac{P_{sed}}{T} + \frac{P_{loss}}{T} \tag{7}$$

The mass balance of P in hydroponics:

$$C_P \cdot M_c = \frac{d}{dt}(C_{PO4-P})V + \frac{P_{plant}}{T} + \frac{P_{loss}}{T} \tag{8}$$

Where, f_N and f_P are the fractions of N and P in fish feed (g N/g feed; g P/g feed), respectively; C_N and C_P are the fractions of N and P in chemical fertilizer (g N/L; g P/L), respectively; M_f is the feeding rate (g/day); M_c is the chemical fertilizer application rate (L/day); C_{TAN}, C_{NO2-N}, C_{NO3-N} and C_{PO4-P} are the concentrations of TAN, NO$_2$–N, NO$_3$–N, and PO$_4$–P in recirculating water (g N/L; g P/L), respectively; V is the volume of recirculating water (L); N$_{plant}$ and P$_{plant}$ are the average N and P assimilated in plant crops at harvest (g N; g P), respectively; N$_{fish}$ and P$_{fish}$ are the average N and P assimilated in fish meat, inner organs, scales, and bones (g N; g P), respectively; T is the production duration (days); N$_{sed}$ and P$_{sed}$ are the N and P in solid waste accumulated in biofilters at the end of each trial (g N; g P), respectively; and N$_{loss}$/T and P$_{loss}$/T are the rate of N loss (g N/day) via denitrification and the rate of P loss via organic P precipitation (g P/day), respectively (B. da S. Cerozi & Fitzsimmons, 2017; Wongkiew et al., 2017). In this study, N$_{loss}$/T and P$_{loss}$/T were unknown and were calculated by subtracting the N and P in fish feed (left side of eq. (5) and eq. (7)) from the rest of known N and P products.

The large variations in fish growth status, plant nutrient removal rate, temperature, pH environment, etc.) made it impossible to identify the precise amount of an individual nutrient throughout a subsystem. For this reason, the forms of N and P in recirculating water were assumed

to be in a dynamic equilibrium over a specific period. Consequently, the mass balances presented the status quo of each component under the steady-state conditions assumed for the production system.

2.8. Estimation of N and P removal rate

Similarly, daily N and P inputs were calculated based on the N and P contents in the fish feed. Based on the results of N and P mass balance, assimilated N and P from fish consumption were calculated by the N and P fractions in fish and solid waste, respectively. The dissolved N and P were then calculated by subtraction of assimilated parts from the fish feed input. The daily removal rate was calculated by the difference between the daily N and P input and the daily N and P concentration in the effluent of hydroponic culture unit in aquaponic and hydroponic systems:

The removal rate of N in aquaponics:

$$N_{rem} = \frac{f_N \cdot M_f - N_{fish} - N_{sed} - \frac{d}{dt}(C_{TAN} + C_{NO2-N} + C_{NO3-N})V}{f_N \cdot M_f - N_{fish} - N_{sed}} \tag{9}$$

The removal rate of N in hydroponics:

$$N_{rem} = \frac{C_N \cdot M_c - \frac{d}{dt}(C_{TAN} + C_{NO2-N} + C_{NO3-N})V}{C_N \cdot M_c} \tag{10}$$

The removal rate of P in aquaponics:

$$P_{rem} = \frac{f_P \cdot M_f - P_{fish} - P_{sed} - \frac{d}{dt}(C_{PO4-P})V}{f_P \cdot M_f - P_{fish} - P_{sed}} \tag{11}$$

The removal rate of P in hydroponics:

$$P_{rem} = \frac{C_P \cdot M_c - \frac{d}{dt}(C_{PO4-P})V}{C_P \cdot M_c} \tag{12}$$

Where, N_{rem} and P_{rem} are the N and P removal rate (%); f_N and f_P are the fractions of N and P in fish feed (g N/g), respectively; C_N and C_P are the fractions of N and P in chemical fertilizer (g N/L; g P/L), respectively; M_f is the feeding rate (g/day); M_c is the chemical fertilizer application rate (L/day); N_{fish} and N_{sed} are the average N in fish tissues and solid waste accumulated by mass balance results (g N), respectively; C_{TAN}, C_{NO2-N}, and C_{NO3-N} ,and C_{PO4-P} are the TAN, NO_2–N, NO_3–N, and PO_4–P concentrations in recirculating water (g N/L), respectively; P_{fish} and P_{sed} are the average P in fish tissues and solid waste accumulated by mass balance results (g N), respectively; V is the volume of recirculating water (L).

2.9. Experimental design and data analysis

The experimental design was a split-plot randomized complete block design (RCBD) with production system and plant species as main plots: tomato-based aquaponics, basil-based aquaponics, lettuce-based aquaponics, tomato-based hydroponics, basil-based hydroponics, and lettuce-based hydroponics; and with research trial (time block) as subplots. Each experiment was

conducted for 3 months in three different time blocks: December through February, April through June, and July through September. Each time block consisted of three aquaponic systems (**Figure 16 A**) and three hydroponic systems (**Figure 16 B**). The data were pooled across time blocks, yielding 3 replicates per system. The number of plant-sample replicates for each system was 8 for cherry tomato and 24 for basil and lettuce. All data were statistically analyzed using JMP® for Windows, Version 13.0 (SAS Institute Inc., Cary, NC). Statistical differences were determined using two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

3. Results

3.1. Water quality in tomato, basil, and lettuce-based aquaponics

Crop species and production system had significant effects on water-quality parameters (**Table 27**). The concentrations of TAN, NO_2–N, NO_3–N, and PO_4–P were significantly different by production system. Aquaponics had significantly ($P < 0.0001$) lower average concentrations of NO_3–N and PO_4–P in recirculating water but significantly ($P < 0.0001$) higher concentrations of NO_2–N and NH_4–N compared to those in hydroponics. The ammonium concentrations were higher than 0.8 mg/L in both hydroponic and aquaponic systems, and the nitrate : ammonium ratio was around 16 for aquaponics and around 80–100 for hydroponics (**Table 27**).

Although there were no significant differences in the average concentrations of TAN, NO_2–N, NO_3–N, and PO_4–P within the systems (**Table 27**), the concentrations changed dramatically over time and varied by the crop (**Figure 18**). Such changes in water chemical environment reflected nitrification processes occurring from the relative new (Phase I) to mature (Phase III) phases of aquaponic system development (**Figure 18 A, B, and C**). During the phase I, TAN concentrations gradually increased, which was followed by nitrite concentration increases up to 19 mg/L. During phases II and III, TAN concentration was initially higher than 4 mg/L then gradually decreased to nearly 0 mg/L, but nitrite concentrations were in the range of 7 to 10 mg/L. Nitrate concentration gradually increased over time and ranged between 20 and 45 mg/L during the phase III.

Tomato-based aquaponic systems showed relatively lower TAN and NO_3–N during phase II than did basil- and lettuce-based aquaponic systems, indicating that a tomato-based aquaponics system had better water quality than other crop species-based systems. Meanwhile, PO_4–P concentration increased quickly in phase I, then maintained at 9 mg/L during phase II and III, regardless of crop (**Figure 18 D**).

Aquaponic systems had significantly lower DO and EC level and higher pH and temperature than did hydroponic systems as a result of combined production of fish and plant crops (**Table 27**). Nevertheless, DO values were well above 5 mg/L, which was sufficient oxygen for growth of fish and plant crops (Rakocy et al., 2012). There were interactions between aquaponic and hydroponic systems for DO, temperature, and EC value ($P < 0.01$). Crop species had significant effects on DO, temperature, and EC value in aquaponic systems, but not in hydroponic systems. Lettuce-based

aquaponics showed significantly higher DO, water temperature, and EC value than did tomato-based aquaponics.

Depending on crop species, Aquaponic systems showed 100 to 400% higher water consumption than did hydroponic systems due to the simultaneous production of fish and plant crops (**Table 28**). Each tomato-based aquaponic system consumed 50 and 30% more water than basil- and lettuce-based systems, respectively. Each tomato plant consumed 300-460%and 1010-2020% more water than basil and lettuce, respectively, partly due to larger leaf area and higher transpiration rate of tomato (**Table 30**).

3.2. Fish growth and yield in tomato, basil, or lettuce-based aquaponics

No fish mortality was observed during the study. Fish yields were affected by different crop species in aquaponic systems (**Table 29**). Despite the same amount of fish feed applied over a 90-day production period, tomato-based aquaponics systems had the highest fish-biomass increment (yield) and the lowest fish-conversion ratio (FCR). Contrarily, lettuce-based aquaponic systems showed the lowest fish-biomass increment and yield and the highest FCR. These results could be due to the better water quality in tomato-based aquaponics systems (**Table 27; Figure 18**).

3.3. N and P removal rate by plant crops in aquaponics and hydroponics

The daily N removal rate was calculated by the difference between the daily N input and the daily N concentration in the effluent of hydroponic culture unit of aquaponics and hydroponics as described in 3.8. In aquaponics, daily N-removal rate by tomato was significantly higher than that by basil and lettuce (**Figure 19 A, B**). Meanwhile, daily N-removal rate by crops grown in hydroponics followed the decreasing order of tomato> lettuce> basil. There was no significant difference in daily N removal rate by tomato between the systems. In general, daily N removal rate by basil and lettuce was higher in aquaponics than in hydroponics.

Similarly, the daily P-removal rate was calculated as the difference between daily P input and daily P concentration in the effluent of hydroponic culture unit of aquaponics and hydroponics as described in 3.8. Daily P-removal rate showed a different pattern from daily N-removal rate (**Figure 19 C, D**). In aquaponics, plant species did not have significant effects on daily P-removal rate. In hydroponics, daily P-removal rate of lettuce was significantly higher than those of tomato and basil. For tomato and basil, daily P removal rate was higher in aquaponics than in hydroponics, but that of lettuce was higher in hydroponics than in aquaponics.

3.4. N mass balance in aquaponics and hydroponics

Production system significantly affected total N distribution (**Figures 20 A, B; and 21 A, B**). In aquaponics, 30 to 40% of N derived from fish feed was assimilated into crop biomass; 21 to 24% to fish body; and 9 to 17% to plant crops (**Figure 20 A**). However, 60 to 70% N was lost to the environment by aquaponic systems; 14 to 20% N remained in the wastewater; 45 to 50% N was released to the atmosphere; and 0.7 to 2% as solid waste. The N-assimilation rate of crops in aquaponic systems ranked as tomato (16.6%) > basil (11.0%) > lettuce (9.4%) in decreasing order,

while N loss via wastewater, solid waste, and denitrification followed the decreasing order of lettuce (69.5%) > basil (67.7%) > tomato (59.2%) (**Figure 20 A**).

In hydroponics, 14 to 25% of N from chemical fertilizer was assimilated into crop biomass production, while 7 to 15% N was lostin wastewater. However, 69 to 78% of N was lost into the atmosphere through denitrification (**Figure 20 B**). Similar to aquaponics, the order of N-assimilation rate by crops in hydroponic systems was tomato (24.3%) > basil (15.5%) > lettuce (13.5%) in decreasing order, while the N loss via wastewater and denitrification followed the decreasing order of lettuce (89.1%) > basil (86.4%) > tomato (78.6%) (**Figure 20 B**). Comparisons of the absolute values of N distribution showed that N loss as atmospheric N release was 2 times higher from hydroponics than from aquaponics (**Figure 21 A, B**).

In terms of total N distribution in plant tissue, different distribution patterns were observed depending on crop (**Figure 22 A, B**). Most N was allocated to the leaves of basil and lettuce regardless of the production system, but tomato showed a different pattern (**Figure 22 A, B**). In both systems, N distribution was leaves > roots in lettuce; leaves > stems > roots in basil. Tomato grown in aquaponics assimilated similar amounts of N into fruits (36%) and leaves (34%). However, tomato grown in hydroponics assimilated N more into leaves (52%) than into fruits (23%).

3.5. P mass balance in aquaponics and hydroponics

Production system significantly affected total P distribution (**Figures 20 C, D; and 21 C, D**). In aquaponics, 46 to 62% of P derived from fish feed was assimilated as crop (fish and plant) biomass: 35 to 45% as fish body, and 11 to 25% to plant crops (**Figure 20 C**). However, 38 to 54% P was lost to the environment in aquaponic systems: 22 to 28% P remained in the wastewater, 2 to 7% was wasted as fish solid waste, 8 to 25% P in the aquaponic solution was unavailable, possibly in the form of suspended particles (not measured in this study). The order of P assimilation rate by crops in aquaponic systems was tomato (25.2%) > basil (16.4%) > lettuce (11.0%), while P loss possibly via precipitation of phosphate salts followed the order lettuce (24.5%) > basil (8.2%) > tomato (8.0%) (**Figure 20 C**).

In hydroponics, 11 to 21% P from chemical fertilizer was assimilated into crop biomass, whereas 52 to 74% P was maintained in the water. However, 15 to 27% of total P likely was lost via precipitation (**Figure 20 D**). Similar to aquaponics, the order of P assimilation rate by hydroponic crops was tomato (21.3%) > basil (13.5%) > lettuce (10.9%), while P loss via precipitation was tomato (27.0%) > basil (22.3%) > lettuce (15.0%) (**Figure 20 D**). Comparing the absolute values of P distribution showed that P loss was significantly higher in hydroponics than in aquaponics, except for aquaponic lettuce, which had 2 to 3 times higher P loss compared to that in hydroponics (**Figure 21 C, D**).

Similar to total N distribution, total P showed different distribution patterns depending on the crop (**Figure 22 C, D**). Most P was allocated to the leaves of basil and lettuce regardless of production system, but tomato showed a different pattern (**Figure 22 C, D**). In both production systems, P distribution was highest in the order leaves > roots for lettuce; leaves > stems > roots for basil.

Aquaponic tomato assimilated similar amounts of P into fruit parts (39%) and leaf parts (33%), whereas hydroponic tomato assimilated P primarily into leaf parts (44%) and secondarily into fruit parts (23%).

3.6. N- and P-use efficiency in aquaponics and hydroponics

In aquaponics, N-use efficiency (NUE) for fish, edible plant parts, and whole plants ranged from 0.21 to 0.24, 0.06 to 0.08, and 0.09 to 0.17 g/g, respectively (**Table 31**). In hydroponics, NUE for edible plant parts and whole plants ranged from 0.06 to 0.13 and 0.14 to 0.24 g/g, respectively. Although NUE was higher for hydroponics than for aquaponics when only edible plant parts or whole plants were considered, NUE for aquaponics was higher than for hydroponics when both whole plants and fish were considered. The NUE of entire aquaponic systems was in range of 0.30 to 0.41 g/g, which was 2 times higher than for hydroponics (0.14 to 0.24 g/g).

P-use efficiency (PUE) was higher in aquaponics than hydroponics even when only edible parts were considered (**Table 31**). In aquaponics, the PUE for fish and edible plant parts was 0.35 to 0.45 g/g and 0.10 to 0.11 g/g, respectively. In hydroponics, the PUE for edible plant parts ranged from 0.03 to 0.09 g/g. When whole plants and fish were considered together in aquaponics, PUE of the whole system (0.46 to 0.61 g/g) was 4 to 5 times higher than for hydroponics (0.10 to 0.14 g/g).

Regardless of crop species, WUE of the whole system in aquaponics was significantly lower than hydroponics (**Table 31**). WUE was not affected by crop species.

4. Discussion

4.1. N and P nutrient concentration in aquaponics and hydroponics

Average nitrate concentrations in aquaponic aquaculture tank and hydroponic fertilizer were 33.5 and 82.3 mg/L, respectively (**Table 27**). In general, N-removal rate was significantly higher from aquaponics than hydroponics, which was 1.1, 2.0, 1.3 times higher for tomato-, basil-, and lettuce-based aquaponics than for hydroponics (**Figure 19**). Our study demonstrated that the aqueous environment of aquaponic systems is not favorable for plant crop production, particularly due to high ammonium concentration (over 0.8 mg/L) and nitrate:ammonium ratio ($\approx$16), suggesting a potential negative impact on plant growth. This is particularly true for young aquaponic systems when nitrifiers are not fully established. Ammonium inhibits nitrate uptake, but that is a highly variable depending on the type of crop (Gessler et al., 1998; Grattan & Grieve, 1999). For example, nitrate uptake by the roots was strongly inhibited in herbaceous plants (Lee & Drew, 1989) and woody species (Kreuzwieser et al., 1997) in the presence of ammonium. Strong inhibition was found for citrus growth at an nitrate:ammonium ratio of 4, or an ammonium concentration of 0.4 to 1.2 mg/L (Serna et al., 1992). In contrast, ammonium has a positive effect on nitrate uptake in ash and oak (Stadler, Gebauer, & Schulze, 1993).

P is a key nutrient for energy metabolism and biosynthesis of nucleic acids and membranes (Marschner, 2011). The average P concentrations in aquaponic and hydroponic water were 8.8 and

118.6 mg/L, respectively (**Table 27**). P removal rate was 1.2 and 1.3 times higher for tomato- and basil-based aquaponics than hydroponics, but P removal rate was 1.2 times lower for lettuce-based aquaponics than hydroponics, indicating P removal ability of different plant species was affected by production system (**Figure 19**). The P concentration in the soilless system was adequate (>1.9–2.8 mg/L) for the growth of most plants (e.g., clover, tomato, petunia, lantana, and flat weed) as long as P is constantly available to the root zone (Asher & Loneragan, 1967; Kim & Li, 2016; Kim, Lynch, & Brown, 2008). Unlike soil-based systems, P concentration can be considerably lowered in water-based systems becasuse P mobility is not restricted in such systems. Therefore, a zero-discharge aquaponic system can provide enough P nutrient for crops if no external factors interfere with P availability (Chapter 2, this dissertation).

4.2. Plant N and P allocation in aquaponics and hydroponics

N loss followed an decreasing order of lettuce (77.6%) > basil (69.6%) > tomato (68.7%) (**Figure 20 B**), which may be a result of the high application of N in hydroponics beyond crop needs.

Tomato, basil, and lettuce were used as model crops based on different growth characteristics, edible parts, and nutrient requirements. This approach allows analysis of the effects of crop species on N and P mass balance. Although some nutrient deficiency has been reported in aquaponic systems such as iron (Rakocy et al., 2012), and calcium and magnesium (Chapter 2, this dissertation), we did not provide nutrient supplements in aquaponics during this study, because our previous study showed that aquaponic crops can perform well even without nutrient supplements, if aquaponic systems are properly managed (Yang & Kim, 2019).

In this study, tomato plants displayed different nutrient-distribution strategies, depending on the production system (**Table 27**). For example, hydroponically grown tomato assimilated N primarily into leaves and secondarily into fruit. In contrast, more than 70% of assimilated N was evenly allocated to the fruits and leaves of aquaponically grown tomato. This is partly due to nutrient limitations in aquaponic solution, which contained 20% and 60% lower N and P than did hydroponics. N is first assimilated into leaves, and then transported to the fruit (Tanemura et al., 2008), implying that such a N-allocation pattern allows higher production capacity in aquaponics, in which plants may experience mild N limitation. In our study, aquaponically grown basil showed slightly higher N allocation to the roots and leaves and a higher ratio of root-to-leaf N (16:74 or 0.22) compared to hydroponically-grown basil (13:73 or 0.18) (**Figure 22 A, B**), suggesting the N-allocation strategy for optimizing total plant growth with limiting N (Ohnmeiss & Baldwin, 1994). Similarly, aquaponic-grown lettuce also showed lower N allocation to leaves but higher N allocation to roots than for hydroponic-grown lettuce, confirming that plants grown in aquaponics develop an allocation strategy to enhance N uptake by enhancing root development (**Figure 22 A, B**).

For P, tomato showed a similar allocation pattern as for N, regardless of production system. Such results indicate that tomato plants in aquaponics may have experienced mild P limitation (60% lower P than hydroponics), thereby developing a P allocation strategy to achieve higher production capacity. However, P allocation in lettuce was not affected by production system nor P supply, which could be explained by the limited P requirement of lettuce (P-efficient when P available

concentration was higher than 3 mg/L) (Buso & Bliss, 1988). Compared to N allocation, basil allocated more P to stems than to roots, which might have led to a lower P allocation to leaves, indicating different allocation strategy for N and P. However, similar to results for N, aquaponicgrown basil also allocated more P in the leaves than in hydroponics, which indicates positive effects of aquaponic wastewater on plant growth similar to an organic manure, in which Anwar et al. (2005) found crop productivity and quality were improved by farm yard manure and vermicompost.

4.3. N distribution and loss in aquaponics and hydroponics

N distribution in an aquaponic system can be affected by crop species, system design (Dabach, Shani, & Lazarovitch, 2015), feed quality, feeding management (Endut et al., 2014), length of the experiment (Groenveld et al., 2019), and measurement methods. This study and previous studies showed that using different fish species did not have significant effects on N distribution in the system, but system design and management could play an important role (**Table 32**).

Most aquaponic studies discharged 2–20% wastewater to maintain better water quality for fish, while 0% discharge systems are known to produce lower fish yield. In our study, N assimilated by fish accounted for more than 20% of N input from fish feed, which was consistent with N-budget data reported in traditional aquaculture research using tilapia (Christie, 2014) and carp (Siddiqui & Al-Harbi, 1999), indicating that the fish in our study received good nutrition despite the use of zero-discharge systems. Plant tissue in aquaponics assimilated 9 to 17% of N input, and these values were similar to previous reports (Fang et al., 2018; Wongkiew et al., 2017). Some other aquaponic research reported extremely low crop N assimilation ranging from 0.4 to 0.5% relative to fish N assimilation (18.7 to 20.0 %) (Delaide et al., 2017; Jaeger et al., 2019). This may be due to undesirable plant-growth conditions (low light, dissolved oxygen, etc.) during their study. Solid waste losses accounted for only 1–2% of N input, which was similar to the value reported by Wongkiew et al. (2017). However, a few studies reported 2 to 5 times higher values of solid waste loss than what were presented here (Delaide et al., 2017; Groenveld et al., 2019; Jaeger et al., 2019). This discrepancy may have been caused by variations in system design (size of solid waste tank, flow rate) and management (frequency of solid waste collection, water-discharge rate) among the studies (**Table 32**).

About 20% of N was retained in aquaponic wastewater in our study, which was consistent with the results in other studies (Delaide et al., 2017; Groenveld et al., 2019; Jaeger et al., 2019; Wongkiew et al., 2017). This means that N is not a limiting factor for production of fish and plant crops in aquaponic systems. A more efficient feeding-management strategy should reduce this portion of N waste to improve the efficiency of aquaponic systems.

Zero-discharge aquaponic systems are characterized by high N loss of over 40% during a production season (Fang et al., 2018; Wongkiew et al., 2017), while aquaponics systems with daily water discharge rates of 10 to 20% had a significant reduction in N loss (<10%) (Groenveld et al., 2019; Jaeger et al., 2019). Daily water discharge in aquaponic systems could enhance removal of suspended solid and some denitrifiers, so that could prevent sludge thickening and alleviate denitrification process, which has been determined to contribute to a reduction in the overall level

of inorganic nitrogen and phosphorus loss (van Rijn, 2013). This un-accounted-for N in aquaponic systems was most likely due to gas emission from denitrification, but not from NH_3 volatilization (Thoman et al., 2001) which is more likely to happen when pH is higher than 8 (Koottatep & Polprasert, 1997). The pH in our study was maintained at around 7. The higher denitrification rate in zero-discharge aquaponic systems could be due to unsuitable biofilm medium design (Takács et al., 2007; Watanabe, Masuda, & Ishiguro, 1992), the anoxic conditions in biofilters (Boxman et al., 2018), or any oxygen-depleted zone (van Rijn, 2013). Setting up oxygen generators and improving the efficiency of water pumps could be a common method to fix the anoxic issue. When comparing designs focusing on anoxic zones, biofilm medium designs have received more attention. Our study used K1 bio-media in the biofilter tanks, which is a round shape, hollow plastic medium and most widely used for aquaponic studies. This hollow design promoted nitrification on the surface, but denitrification internally (Torresi et al., 2016; Watanabe et al., 1992). This kind of bio-medium may be perfect for an aquaculture system where both nitrification and denitrification could reduce toxic N for fish, but obviously not suitable for an aquaponics system where only nitrification is desired. This flaw in system design will be important to rectify in future experiments. Finally, some portions of N loss may be due to trapped N on tank wall, bottom of grow beds, and biofilter tank (Delaide et al., 2017) and this might have caused an underestimation of solid waste. However, there was non-collected solid waste found in our system in negligible portion as they were present as particles in wastewater, so this part of un-accounted N could be omitted in our study.

It is surprising to find that the total N loss in hydroponic systems ranged from 76 to 87% (**Figure 20 B, D**). Nearly 7-15% N was lost in the wastewater and 69-78% N was lost presumably by denitrification and adsorption into the substratum and biofilm (Delaide et al., 2017; Wongkiew et al., 2017). Such high N loss in hydroponics is mainly assumed due to the high N fertilizer input beyond the crop needs and possibly due to the result of lower pH and slightly higher dissolved oxygen level. It was reported that "semianaerobic" conditions (0.35–5% O_2 depending on nitrifying bacterial strains) may push N transformation in the direction of denitrification (Knowles, 1982). Such high N loss in hydroponics can cause significant environmental issues. It should be noted that the hydroponic solution was recycled for 90 days. In commercial hydroponic operation, however, spent nutrient solution is discharged every 2 weeks or after each harvest (one month). Considering commercial practices, the wastes generated from hydroponic operations could be even higher than what was presented in this research-scale study. While hydroponic systems are known to be the more efficient production system compared to conventional field production, our results demonstrate that current management practice is not sustainable. Significant changes in current nutrient-management practices are needed to further improve the system efficiency of hydroponics.

4.4. P distribution and loss in aquaponics and hydroponics

There is very limited information on P budget available in the literature. In our study, the P mass balance indicated 76 to 92% of P recovered from the fish-feed input, which was 18 to 26% higher than the values for hydroponics (except lettuce), demonstrating that aquaponic systems can improve P use. Our results showed that about 35 to 45% of P was retained in fish, which was consistent with the values reported in previous studies (Seawright et al., 1998; Suloma, Mabroke,

& El-Haroun, 2013), indicating that P nutrition for fish was suitable in our study. Plant tissues assimilated 10 to 25% P from fish feed, which was 3 to 4% higher than the values in hydroponic system (except for lettuce). The fish waste accounted for 2 to 7% of P from feed input, which was higher than the 1.3% in aquaculture sludge (Ebeling & Timmons, 2002), indicating limited organic P degradation in solid waste in aquaponics. This could be improved by introducing P-solubilizing bacteria under P limiting conditions (B. da S. Cerozi & Fitzsimmons, 2016; Daughton & Cook, 1979; Kortstee et al., 1994). However, P was not limited in our system, and therefore using P-solubilizing bacteria may not be practical.

Unlike nitrogen, P is not found in a gaseous form, mass balance analysis was performed for each compartment of the system, so a well balanced nutrient budget was expected. However, our mass-balance calculations demonstrated that 8 to 25% of P was missing in the aquaponic system. This might be due to P loss in the suspended solids and sludge or due to underestimation of the quantity of solid waste. Similarly, (Cerozi & Fitzsimmons, 2016; Jaeger et al., 2019) showed inaccurate P budget due to inaccurate measurement and sampling as particles or sludge were suspended and not collected by the solid waste tank. This underestimation could be rectified by collecting all trapped P on tank walls, bottom of grow beds, and biofilter tank, although this was not possible in our study.

Importantly, hydroponic systems showed three times higher P loss as wastewater and nutrient loss due to P precipitation compared to those in aquaponics (**Figure 21 C, D**). P is unavailable at pH<6.5 (Hartikainen & Yli-Halla, 1996) or when precipitated with Fe, Ca, and Mg (Gahoonia, Claassen, & Jungk, 1992). Therefore, this unaccounted P loss may be a result of high Ca and Mg concentrations in hydroponics (146and 40 mg/L, respectively), which might have caused the precipitation of phosphate salts (Van den Berg & Rose, 1959). Tomato-based aquaponics showed significantly lower P loss compared to tomato-based hydroponics, which may be as a result of too much P application in hydroponics beyond the crop needs. The inorganic forms of phosphate will generate chemical precipitation when combined with a coagulant (calcium, magnesium and iron, etc.), which commonly happens when the concentration of soluble phosphate is higher than 1.0 mg/L (Graziani, 2006). The required chemical coagulant dose for P precipitation is related to the liquid P concentration. For example, a dose of 1 mole of magnesium or iron per mole of P is sufficient to precipitate most P element when soluble phosphate concentrations above 2 mg/L (Graziani, 2006; Stratful, Scrimshaw, & Lester, 2001). In addition, the pH value is an important factor for chemical precipitation of P when combined with Mg or other salts, as the solubility of their precipitates vary with pH (Graziani, 2006; Stadler et al., 1993). For example, P precipitation is most efficient in the pH range of 9.0 to 9.5 for magnesium and of 6.5 to 7.5 for ferric salts. Thus, over-application of P in hydroponics solution, which commonly contain sufficient coagulant ions, lead to increment of P precipitation or loss.5.5. N- and P-use efficiency in aquaponics and hydroponics

Nutrient-use efficiency is an indicator of the efficiency of an agricultural production system. The NUE by whole plants was lower for aquaponically grown tomato, basil, and lettuce than for hydroponically grown ones by 41%, 36%, and 56%, respectively. However, after taking fish into

account, NUE for tomato-, basil-, and lettuce-based aquaponic systems was higher than for hydroponics at 70.8%, 113.3%, and 114.3%, respectively (**Table 31**).

Similarly, the PUE of aquaponics was significantly higher than that of hydroponics by 233%, 38%, and 11% for tomato-, basil-, and lettuce-based systems, respectively, even when only the edible plant parts were included. Furthermore, after taking fish into consideration, PUE for tomato-, basil-, and lettuce-based aquaponics was higher than for hydroponics by 336%, 369%, and 360%, respectively (**Table 31**). These results indicate that aquaponics is more efficient in P use relative to hydroponics. More efficient P nutrient-management practices should be developed to improve PUE in hydroponic systems.

Nutrient-budget results varied among aquaponic studies due to different systems management such as solid-waste-clean frequency, fish-stocking density, crop-planting density, and wastewater-discharge rate (Jaeger et al., 2019). Our NUE values were similar to those reported by Fang et al. (2017), Hu et al. (2015), and Zou et al. (2017). In addition, our PUE values were similar with (Jaeger et al., 2019), but higher than those reported by (Delaide et al., 2017). The variations existed among the studies mostly caused by system errors that cannot be prevented, especially for a complex system like aquaponics.

In the aquaponic system, a single performance indicator such as NUE or PUE could be misleading in the assessment of the efficiency and effectiveness (Ball & Wilkinson, 1994; Fixen et al., 2015). Having two indicators in this study can make this evaluation of system efficiency more reliable. In spite of that, other nutrient performance indicators may be necessary in future research (Norton, Davidson, & Roberts, 2015) to evaluate efficiency, and environmental impact of aquaponic systems, which will contribute to the development of sustainable management practices for aquaponic systems.

5. Conclusions

The present study examined nitrogen- and phosphorus- use efficiency (NUE; PUE) of aquaponic and hydroponic systems using a mass-balance approach. The NUE and PUE of the aquaponic system were, respectively, 71 to 114% and 336 to 369% higher than those for hydroponics. N loss from aquaponic systems via solid waste, wastewater, and denitrification ranged from 59.2 to 69.5%, whereas that from hydroponic systems via wastewater discarded at the end of crop production and denitrification ranged from 75.7 to 86.5%. P loss from aquaponic systems via solid waste, wastewater, and P precipitation ranged from 38.4 to 53.6%, while that from hydroponics systems via wastewater and P precipitation ranged from 78.6 to 89.1%. While the environmental wastes generated from aquaponic systems are significantly lower than those from hydroponic systems, it is apparent that the systems can be further improved by plant- production enhancement. Further, current management practices of N and P application in hydroponics are not sustainable. Plant species had significant influence on N and P removal and mass balance in aquaponics and hydroponics. Tomato-based aquaponics had higher NUE and PUE than did basil- and lettuce-based aquaponics. Mass-balance analysis indicated that hydroponic systems had, respectively, 24.5–27.9% and 66.2–125.0% higher N and P loss than did aquaponic systems, mainly due to extensive nutrient

application rate. Further research is necessary to reduce the N and P loss and to further improve the NUE and PUE of aquaponics and hydroponics.

6. References

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Table 26. Macro- and micro-nutrient compositions and concentrations used in hydroponics solution and fish feed used in aquaponics.

Parameter	Hydroponics[a]		Aquaponics[b]
	Basil/Lettuce	Tomato	(/g Fish feed)
Macronutrient (%)			
Total nitrogen (N)	0.043	0.044	> 6.88
P_2O_5–P	0.093	0.130	> 1.10
K_2O–K	0.035	0.034	0.99
SO_4–S	–	–	0.43
Ca	0.075	0.075	2.25–2.75
Mg	0.039	0.037	0.23
Micronutrient (mg kg^{-1})			
B	2.00	2.75	–
Cu	1.05	0.95	10
Fe	21.00	10.00	40
Mn	1.90	8.00	80
Mo	0.42	0.40	–
Zn	2.10	2.70	153

All the information comes from related company.

"–" means "not included" or no related information.

[a] Nutrient compositions of fertilizer used in hydroponics were calculated based on 1:100 dilution of commercial fertilizer.

[b] Nutrient compositions of fish feed used in aquaponics were calculated based on /g feed per day.

Table 27. Average concentrations of water-quality parameters in tomato-, basil- or lettuce-based aquaponics and hydroponics during production period.

Crop species	Production system	TAN (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	DO (mg/L)	pH	Temperature (°C)	EC (mS/cm)
Tomato	Aquaponics	2.11 a	4.68 ab	32.5 b	8.83 b	7.02 c	6.82 a	25.27 b	0.54 c
	Hydroponics	1.22 a	0.03 b	95.5 a	122.81 a	8.59 a	5.86 b	21.88 c	1.95 a
Basil	Aquaponics	1.91 a	4.98 a	35.4 b	9.09 b	7.05 bc	6.79 a	25.06 b	0.84 b
	Hydroponics	0.88 a	0.04 b	73.9 a	107.42 a	8.61 a	5.81 b	21.66 c	1.97 a
Lettuce	Aquaponics	1.88 a	4.82 a	32.5 b	8.50 b	7.20 b	6.84 a	26.14 a	0.92 b
	Hydroponics	0.79 a	0.04 b	77.5 a	125.53 a	8.56 a	5.83 b	21.83 c	1.96 a
F value		0.46	2.46	29.38	24.15	447.38	563.06	362.65	916.48
P		ns	*	***	***	***	***	***	***
ANOVA									
Crop		ns	ns	ns	ns	ns	**	***	***
System		*	***	***	***	***	***	***	***
Crop × System		ns	ns	*	ns	**	ns	***	***

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05).

Each value in the table is the mean of 30 replicates.

ns,*,**,*** mean no significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 28. Cumulative water consumption of tomato-, basil- or lettuce-based aquaponics and hydroponics and the average water consumption per crop during production period.

Crop species	Production system	Cumulative water consumption (L)	Water consumption (L/crop)
Tomato	Aquaponics	1577 a	100.4 a
	Hydroponics	967 c	72.1 ab
Basil	Aquaponics	1064 b	25.5 b
	Hydroponics	465 d	12.8 b
Lettuce	Aquaponics	1216 b	9.0 b
	Hydroponics	294 d	3.4 b
F value		57.06	6.42
P		***	**
ANOVA			
Crop		***	***
System		***	ns
Crop × System		***	ns

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05).

Each value in the table is the mean of 3 replicates.

ns,*,**,*** means not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 29. Fish-stocking density, fish-feed consumption, fish-biomass increment, and feed-conversion ratio (FCR) in aquaponics.

Parameters*	Tomato-based aquaponics	Basil-based aquaponics	Lettuce-based aquaponics
Initial fish stocking density (kg/m^3)	19.7 a	20.0 a	20.3 a
Harvest fish stocking density (kg/m^3)	27.3 a	26.5 a	25.8 b
Average fish feed (g/day)	89.3	89.3	89.3
Fish feed applied (g/90 days)	3,680	3,680	3,680
Fish biomass increment (%)	38.6	32.5	27.1
Feed conversion ratio (FCR)	1.27	1.49	1.76

* Fish feed consumption is the dry weight, while fish biomass increase is the fresh weight.
The FCR was calculated from the relationship of feed intake and weight gain, by the following formula: FCR = $m_c/(m_f-m_i)$, where m_f=final mass, m_i=initial mass and m_c=amount of food consumed.
Fish biomass increment was calculated from the relationship of final fish mass and initial fish mass.
Each value in the table is the mean of 3 replicates.

Table 30. Plant photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration, and intrinsic water use efficiency (WUE) of crops grown in aquaponics and hydroponics systems. The values presented here are photosynthetic parameters measured at day 60 after transplanting.

Crop species	Production system	Photosynthetic rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Intercellular CO_2 concentration ($\mu mol\ CO_2\ m_{-2}\ s^{-1}$)	Transpiration rate ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Intrinsic WUE ($\mu mol\ CO_2\ mmol^{-1}\ H_2O$)	Leaf area (cm^2)
Tomato	Aquaponics	9.8 a	0.4 a	314.2 a	4.4 a	2.4 c	15356 a
	Hydroponics	9.0 a	0.2 b	292.2 b	3.3 ab	2.9 bc	13055 a
Basil	Aquaponics	10.1 a	0.2 b	283.1 b	3.3 ab	3.6 a	2957 bc
	Hydroponics	9.5 a	0.2 b	300.0 ab	3.2 b	3.1 ab	4430 b
Lettuce	Aquaponics	7.6 b	0.2 b	314.4 a	3.3 ab	2.5 c	2556 c
	Hydroponics	7.1 b	0.2 b	295.3 ab	3.0 b	2.6 bc	2376 c
F value		18.76	10.83	6.89	2.70	10.61	60.20
P		***	***	***	***	***	***
Significance							
Crop type		***	***	***	*	***	***
Production system		ns	***	***	**	ns	ns
Crop type × Production system		ns	**	**	ns	*	*

Means within column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test (α =0.05). Each value in the table is the average of 2 crop species for fast-growing, medium-growing, and slow-growing crops, respectively.

Each crop species consisted of 12 replicates.

ns, *,**,*** mean no significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 31. Nitrogen and phosphorus-use efficiency and integrated water-use efficiency of marketable part and fish meat of different crop species in aquaponic and hydroponic systems.

Crop species	Production system	N use efficiency (g·g⁻¹)				P use efficiency (g·g⁻¹)				Water use efficiency (g·L⁻¹)
		Fish	Edible plant part[a]	Whole plant[b]	Whole system[c]	Fish	Edible plant part[a]	Whole plant[b]	Whole system[c]	Whole system[c]
Tomato	Aquaponics	0.24	0.06	0.17	0.41	0.36	0.10	0.25	0.61	23.43 ab
	Hydroponics	-	0.06	0.24	0.24	-	0.03	0.14	0.14	49.65 ab
Basil	Aquaponics	0.21	0.08	0.11	0.32	0.45	0.11	0.16	0.61	21.31 b
	Hydroponics	-	0.11	0.15	0.15	-	0.08	0.13	0.13	84.65 a
Lettuce	Aquaponics	0.21	0.08	0.09	0.30	0.35	0.10	0.11	0.46	18.15 b
	Hydroponics	-	0.13	0.14	0.14	-	0.09	0.10	0.10	56.43 ab

[a] Edible plant part means marketable tissue, which is fruit part for tomato, leaf part for basil and lettuce;
[b] Whole plant means all plant tissues, which include leaf, stem, root and fruit (if applicable).
[c] Whole system means all crops, which include fish and plant crops.

Table 32. Nitrogen and phosphorus mass balance in our and other aquaponic system studies.

| Fish species	Plant species	Study duration (days)	Discharge rate /System design	N assimilated in (%)				P assimilated in (%)				Reference
				fish	plant	Sediments	Un-accounted	fish	plant	Sediments	Un-accounted	
Tilapia	Tomato	92	0% /DWS[a]	21-24	9-17	0.7-2	45-50	35-45	11-25	2-7	8-25	Teng & Kim, 2019
	Basil	92										
	Lettuce	92										
Tilapia	Pak choi,	32-37	0% /DWS	20	20	3	43	-	-	-	-	Wongkiew et al., 2017
	Lettuce											
Tilapia	Basil	28-35	2–3.6% /DWS	20	0.5	10	52	5	0.5	2	90	Delaide et al., 2017
	Lettuce											
Barramundi	cucumber	40	10% /DWS	32.7	-	5.1	11.1	-	-	-	-	Groenveld et al., 2019
Common carp	Algae	50	0% /DWS	20	15	-	65	-	-	-	-	Fang et al., 2018
	Lettuce	52	20% /DWS	47.6	0.4	4.6	24.6	51.6	0.4	22.5	6.6	Jaeger et al., 2019

[a] Deep-water system.
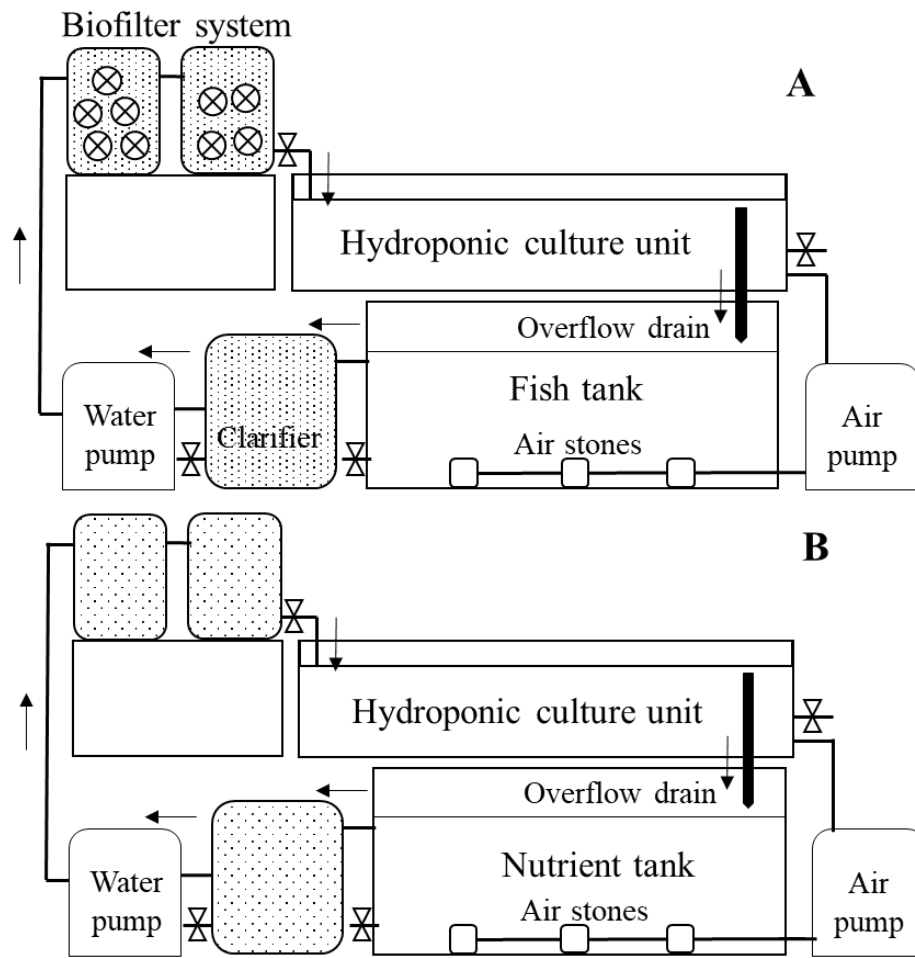
Figure 16. Schematic diagram of experimental units: (A) aquaponic system; (B) hydroponic system.
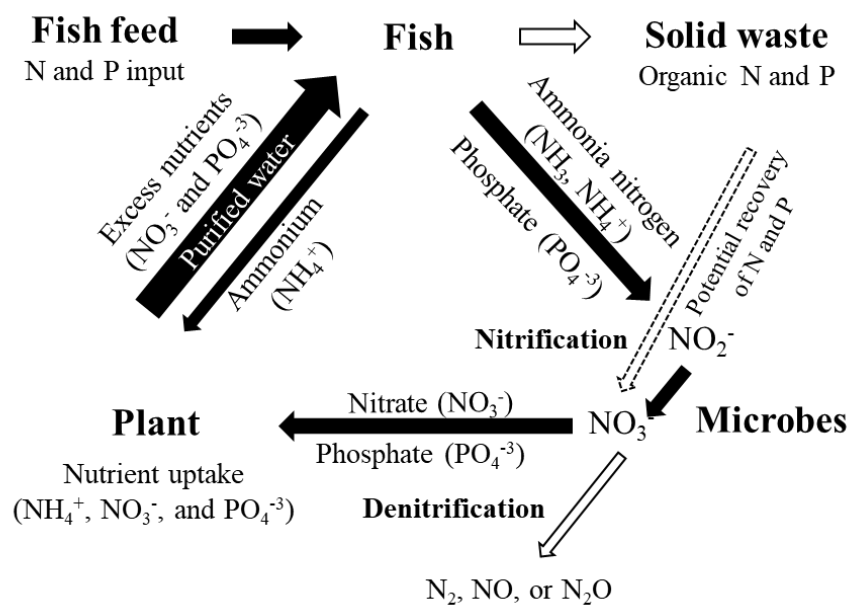
Figure 17. Conceptual diagram for nitrogen and phosphorous source and removal mechanisms in the aquaponic system.
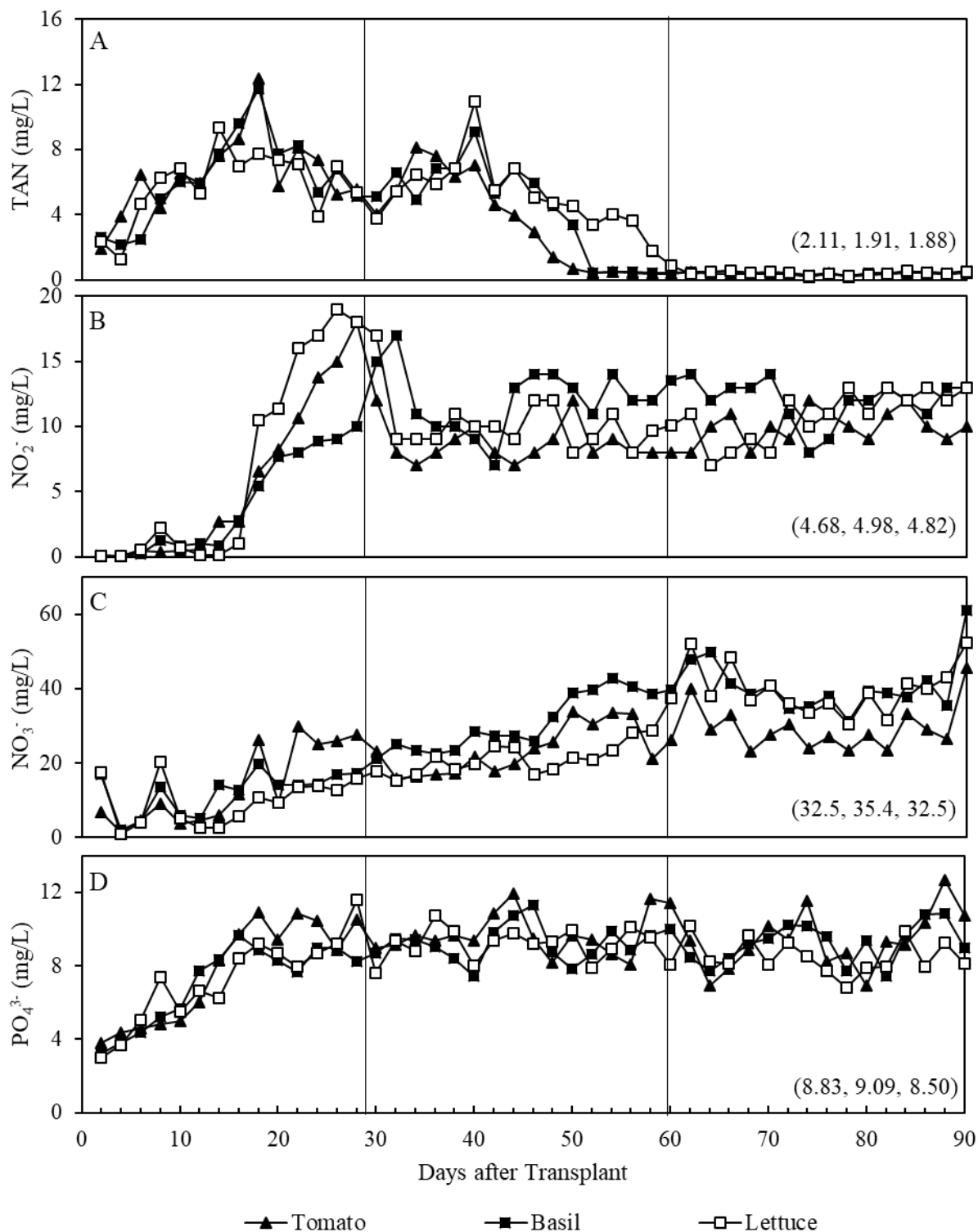
Figure 18. Changes of (A) TAN, (B) nitrite, (C) nitrate, and (D) phosphate concentrations in aquaponic solution during three production periods (Phase I, II, and III) of lettuce and one production period of basil and tomato in a tilapia-based aquaponic system.
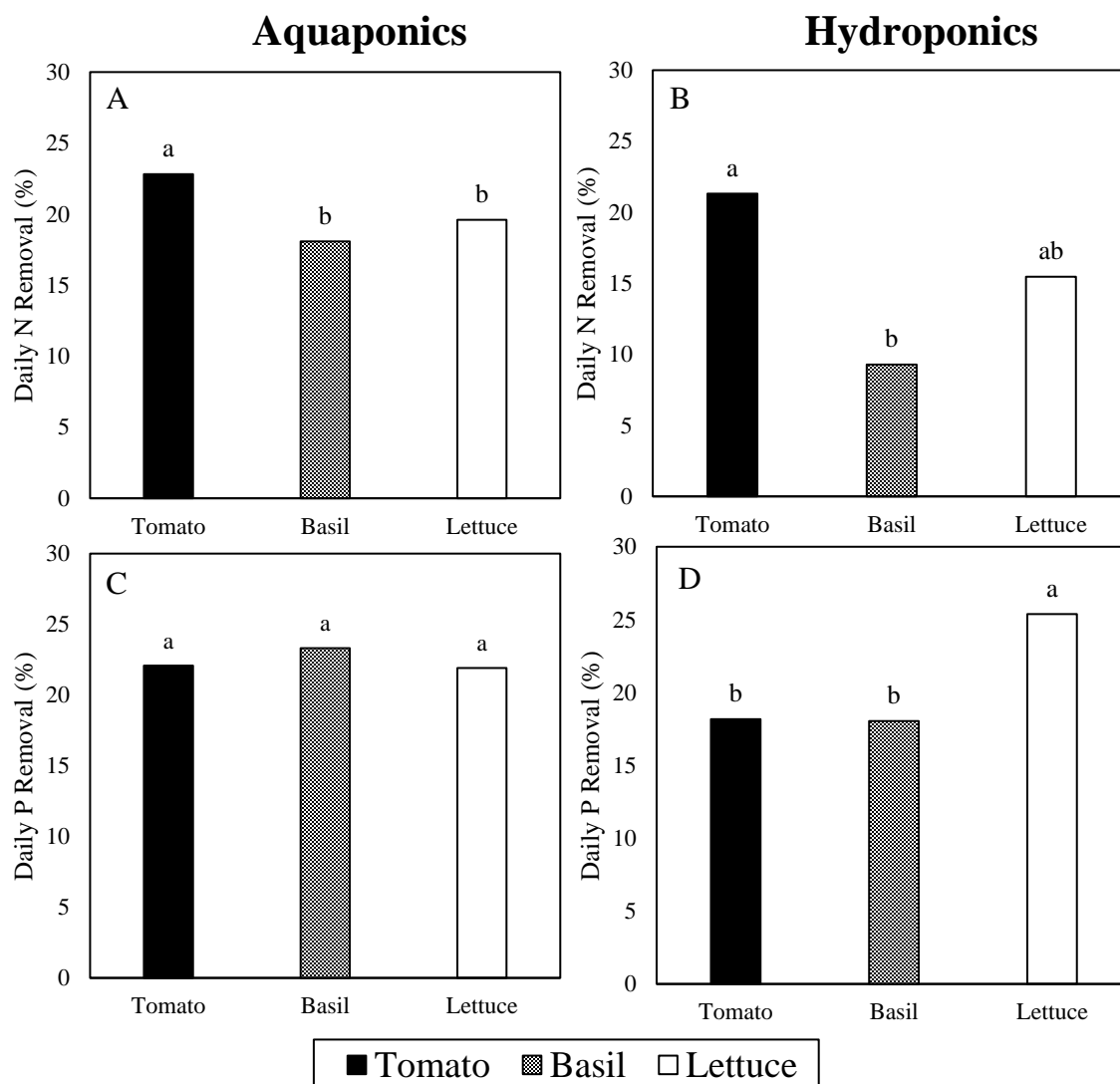
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Figure 19. Daily removal efficiency (%) of N (nitrate) and P (phosphate) from aquaculture wastewater (A, C) or hydroponic fertilizer (B, D) as affected by the production of lettuce, basil, or tomato in aquaponic or hydroponic system during the study period.
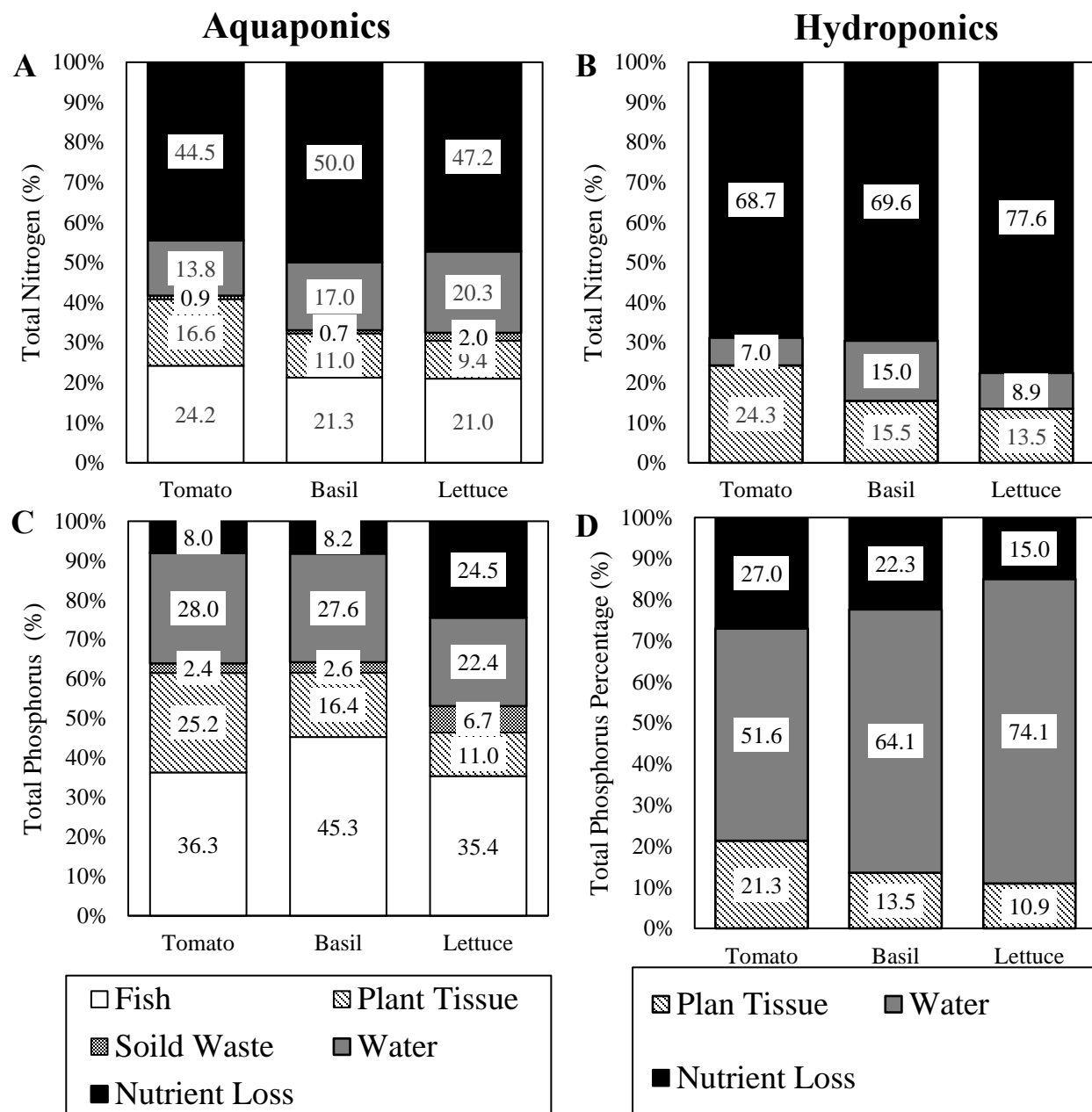
Figure 20. Total Nitrogen and phosphorus distribution in aquaponic and hydroponic systems based on different plant species. (A) & (B) Aquaponics; (C) & (D) Hydroponics.
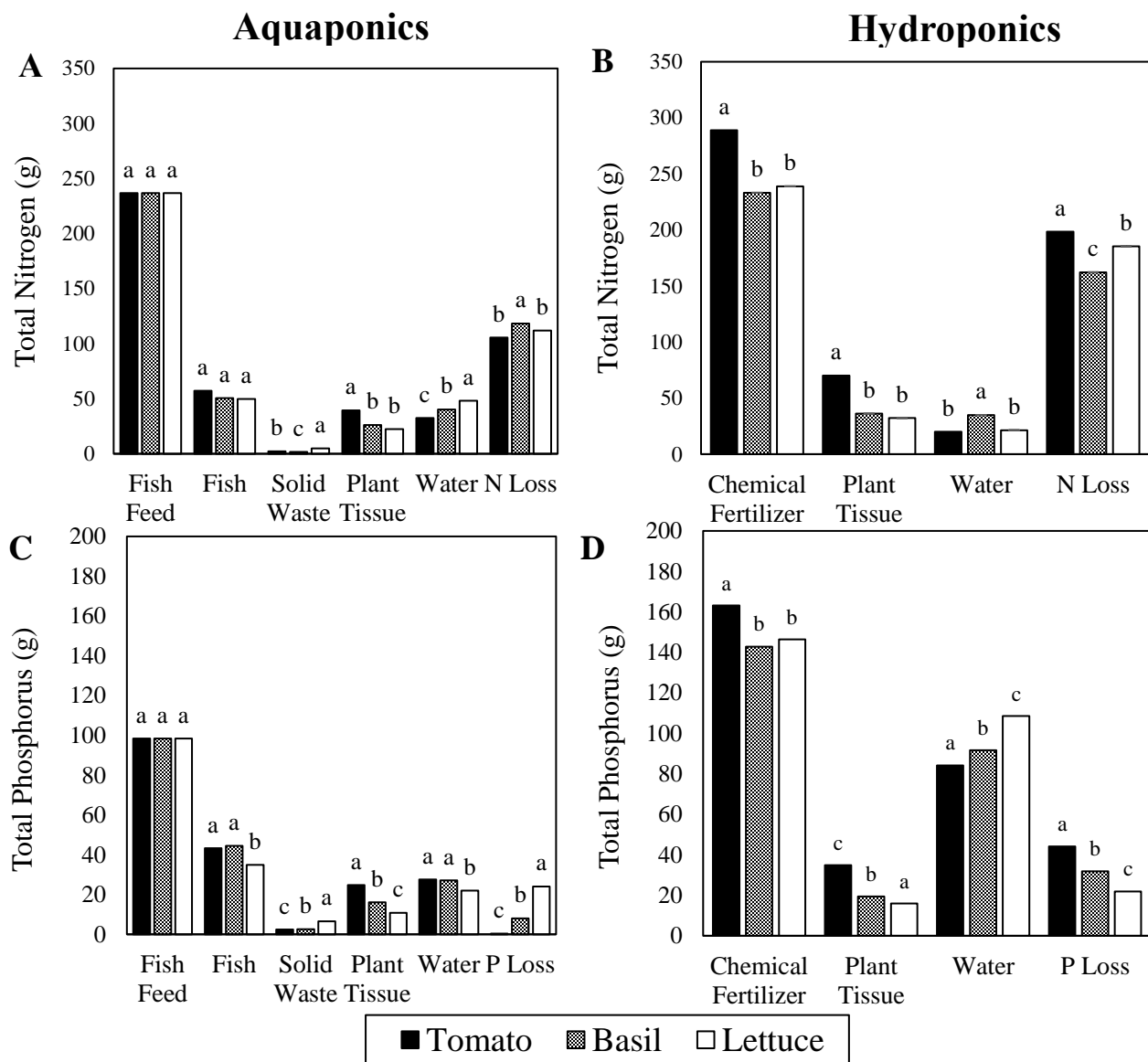
Figure 21. Total Nitrogen and phosphorus content in aquaponic and hydroponic systems based on different plant species. (A) & (B) Aquaponics; (C) & (D) Hydroponics.

Column followed by the same letter are not significantly different based on Tukey's honestly significant difference test (α =0.05).
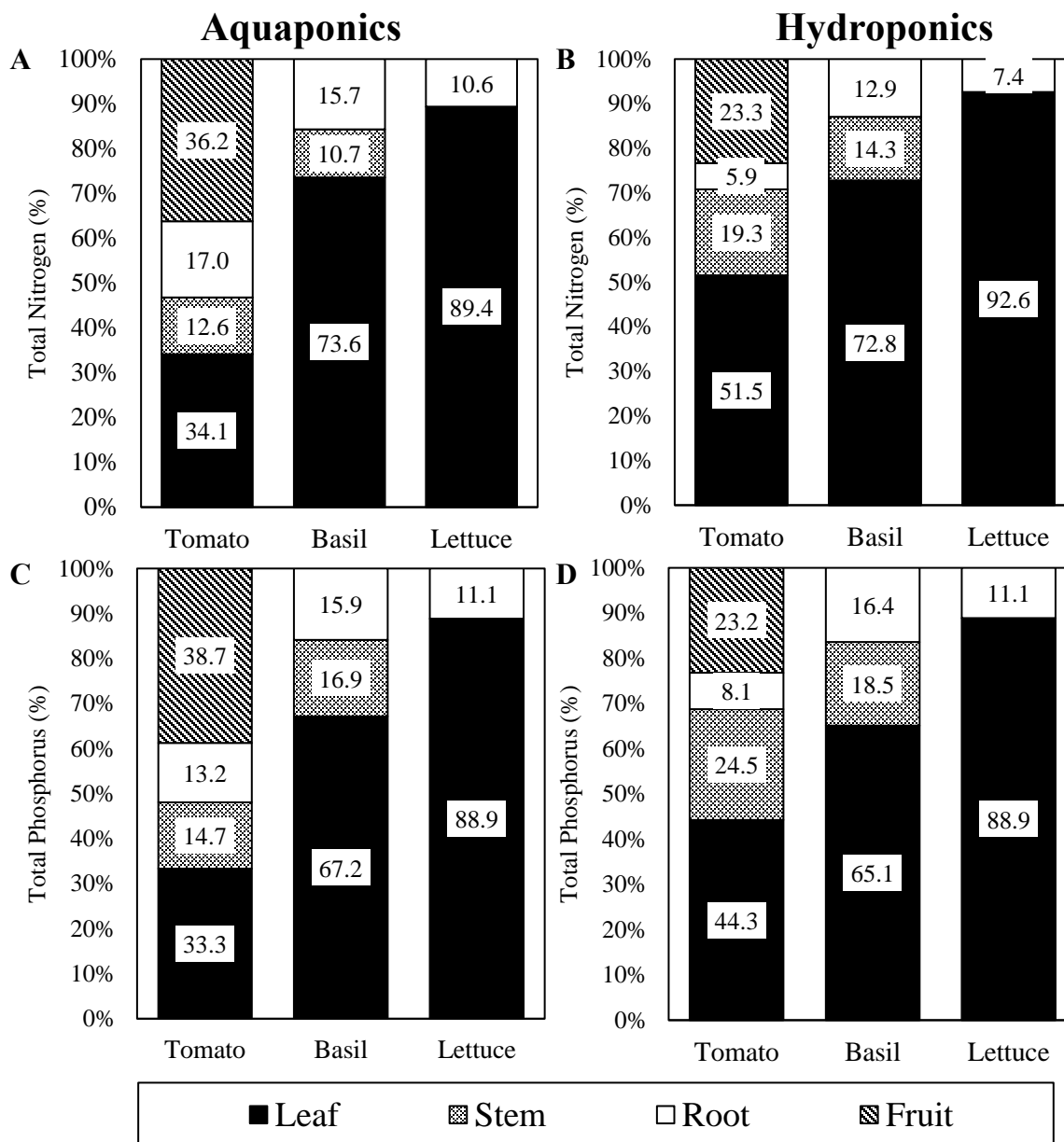
Figure 22. Relative distribution of total nitrogen and phosphorus in plant tissues of tomato, basil, and lettuce grown in aquaponic and hydroponic systems.

(A) Total nitrogen & (C) total phosphorus Aquaponics; (B) & (D) Hydroponics.
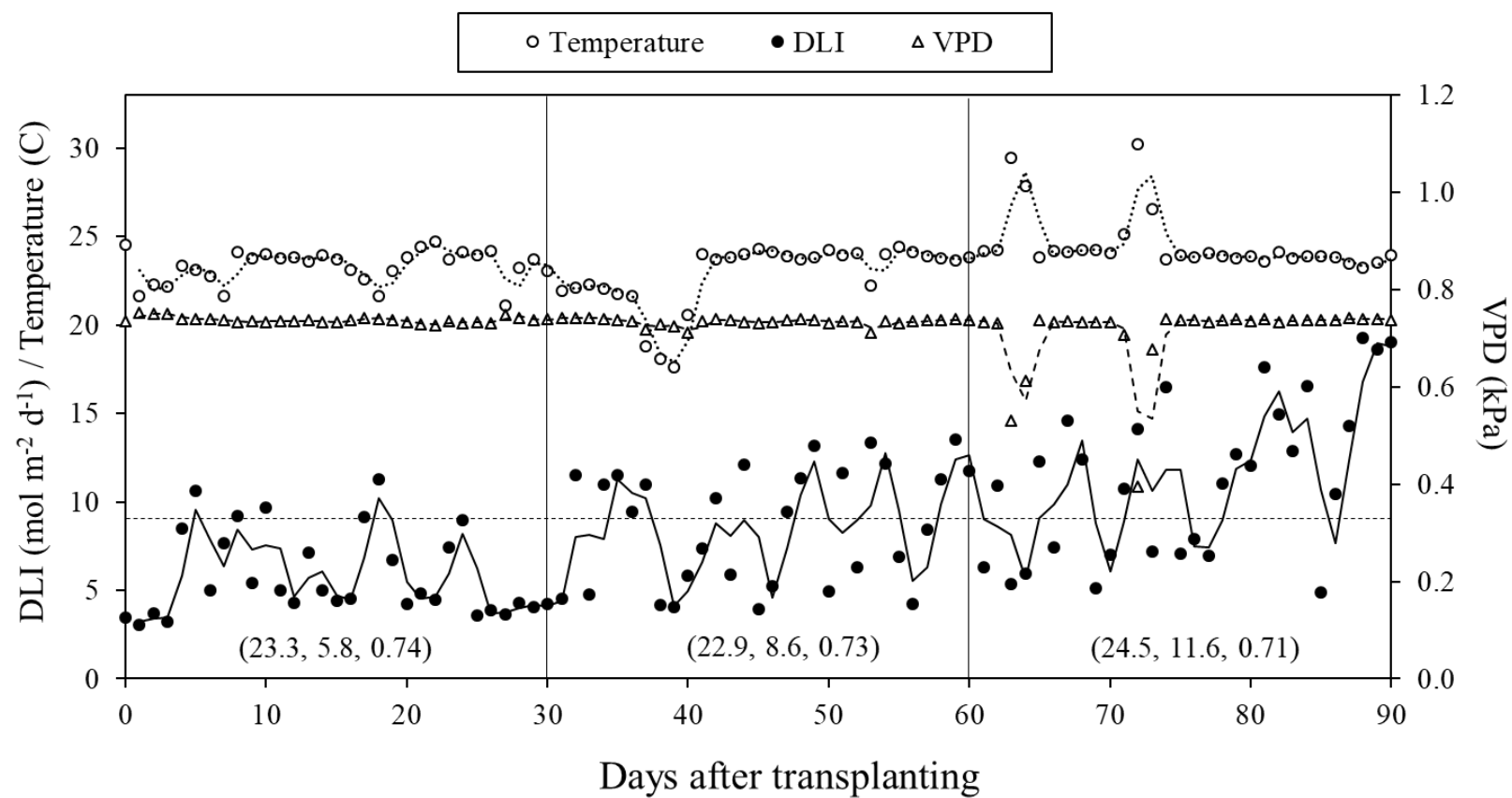
Figure S4. Ambient temperature, daily light integral (DLI), and vapor pressure deficit (VPD) collected in the greenhouse during the experimental period (December through February). The parameters were averaged over the day. The dotted line is the average DLI during entire production period (8.9 mol m^{-2} d^{-1}).