

Optimization of nutrient solution management for leafy greens production in recirculating
hydroponic systems under controlled environments

by

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Abstract

Nutrient solution (NS) and electrical conductivity (EC) management of soilless cropping systems is crucial to optimize the yield and quality of hydroponic crops. The first objective of this thesis was to complete a comprehensive review of current nutrient management strategies in recirculating hydroponic systems, including hydroponic systems design, nutrient formulations, and nutrient management strategies. The review identified critical gaps in knowledge relating to how NS volume in recirculating hydroponic systems impacts nutrient management, along with gaps in knowledge to optimize EC for less-studied leafy greens, such as kale (*Brassica oleracea* ‘Winterbor F1’) and Malabar spinach (*Basella alba* ‘Rubra’), which may have different nutrient requirements compared to lettuce (*Lactuca sativa* ‘Salanova Red Butter’) and basil (*Ocimum basilicum* ‘Large leaf’), which are most frequently studied.

Secondly, two greenhouse experiments were conducted to study the effect of NS volume low (75 L; 2 L per plant) or high (150 L; 4 L per plant) on growth, yield, tissue nutrient concentration; and postharvest quality parameters, including vitamin C, anthocyanins, total phenolics, and antioxidant capacity of four leafy greens species: butterhead lettuce (*Lactuca sativa* ‘Salanova Red Butter’), arugula (*Eruca sativa* ‘Standard’), kale (*Brassica oleracea* ‘Red Russian’), and the emerging crop Malabar spinach (*Basella alba* ‘Rubra’). Results showed that using a low NS volume resulted in higher nitrogen (N) uptake efficiency and lower N retention in the nutrient film technique (NFT) system in both seasons’ trials. NS volume had no impact on growth and yield parameters, but it influenced nutrient composition and quality attributes of leafy greens. Tissue N and potassium (K) in summer and phosphorus (P) and K in fall increased with high volume. Low NS volume reduced nitrate levels in shoots of arugula, lettuce, and Malabar spinach during both seasons, suggesting that lower volume may help minimize

excessive nitrate levels in plant shoots, while kale consistently surpassed recommended safe nitrate levels regardless of treatment. NS volume influenced key postharvest attributes of color, total phenolics, and antioxidant capacity.

Third, in experiments with indoor growth tents, effects of NS EC levels (standard: 1.8 and elevated: 3.6 mS·cm⁻¹) on growth, yield, shoot nutrient concentrations, and postharvest nutritional quality of four leafy green species (basil, kale, lettuce and Malabar spinach) were studied. Plant height and SPAD values were higher with elevated EC across species, with SPAD particularly increased in Malabar spinach. Shoot fresh weight was increased by elevated EC across all species. Shoot and root dry weights were increased by elevated EC, particularly in kale. Tissue analyses revealed high potassium (K) concentration at elevated EC. Standard EC reduced nitrate levels in basil, lettuce, and Malabar spinach within safe range limits, while kale surpassed recommended safe nitrate levels regardless of treatment. Standard EC levels could be a potential strategy to produce K-limited leafy greens to support a low K diet for kidney-diseased patients. Postharvest quality, including color, texture, and nutritional quality, vitamin C, anthocyanin, total phenolics, and antioxidant capacity, was not affected by EC levels. Based on our results, standard EC is optimal for basil and lettuce, while elevated EC may benefit kale and Malabar spinach. For Malabar spinach, high wire production systems should be explored to support its vining growth habit and extend harvest duration to increase yield. This thesis advances the understanding of species-specific responses to nutrient solution volume and EC in recirculating hydroponic systems, providing evidence-based recommendations to improve nutrient use efficiency, crop nutritional quality, and sustainability in controlled environment agriculture.

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Chapter 1 - Review of Current Nutrient Management Techniques for Hydroponic Production

1.1 Hydroponics in Controlled Environment Agriculture

Controlled Environment Agriculture (CEA) allows producers to control environmental factors such as temperature, air movement, humidity, and light levels (Srivani et al., 2019) for crop growth and development. CEA can increase production efficiency, maximize yields, and improve crop quality by optimizing the year-round production environment for predictable plant responses (Gómez et al., 2019; Jensen, 2002). In the United States, CEA operations, including greenhouses, vertical farms and plant factories, and hydroponics and aquaponics facilities, have more than doubled from 1,476 in 2009 to 2,994 in 2019 (USDA, 2024).

Hydroponics, a key component of CEA, is a soilless cultivation method. Originating from Greek words hydro (water) and ponos (labor), hydroponics is the practice of growing plants without soil using nutrient-rich water solutions with or without an inert substrate (Resh, 2022). Hydroponics was introduced by William Frederick Gericke in 1937 at the University of California and emerged as a solution to address soil contamination and provide an opportunity to enhance food production for a growing global population. Hydroponics offers several benefits, such as control over water and nutrient availability to crops, shorter production cycles, and no need for weed control (Bihari et al., 2023; Raviv et al., 2019; Sousa et al., 2024).

1.2 Role of CEA in Urban Food Systems

Over the past decade, urban and peri-urban agriculture has gained attention from urban planners and policymakers. This increasing interest is particularly driven by factors such as increasing urbanization, decline of arable land, and climate change (Benke & and Tomkins,

2017). Global food production will need to be increased by 70% to meet the demand of 10 billion people by 2050 (WHO, 2018). Additionally, 6.5 billion people are expected to live in urban areas where space and resources are limited (Avtar et al., 2019). Together, these concerns have led to calls for a new approach to farming that can improve food security while lowering its environmental impact (McClements et al., 2021). CEA is one of the potential technologies that can intensify food production in urban areas (Glaros et al., 2022). Local production of vegetables and fruits exceeded \$14 billion in North America and is experiencing a 20% annual growth rate (Eaves & Eaves, 2018). Hydroponic systems are increasingly seen as a viable solution to produce fresh, residue-free, high-quality vegetables, which are extremely advantageous for urban markets (George & George, 2016).

The optimization of environmental conditions such as temperature, light, CO₂, and humidity in CEA structures helps to extend the growing season and enables year-round production (Bisbis et al., 2018; Saad et al., 2021). High planting density and continuous production with CEA allow for efficient space utilization, addressing the constraints of land availability in cities (Gómez et al., 2019). Establishing CEA facilities such as greenhouses near urban areas can significantly reduce food miles, help reduce food waste, make local food production more environmentally friendly, and help in food security and safety (Kalantari et al., 2018; Vatistas et al., 2022). Additionally, CEA offers socioeconomic benefits by generating opportunities for skilled labor, stimulating urban economies, and improving community viability (Kozai, 2013).

1.3 CEA Operation Facilities

1.3.1 Greenhouses

Greenhouse horticulture operates in an environment that uses solar energy for heating and photosynthesis (Graamans et al., 2018). Ventilation, heating, and cooling systems in a greenhouse regulate internal climate by managing excess heat or providing warmth as needed (Kittas et al., 2017). This regulation allows crop production in unfavorable climates (Vatistas et al., 2022). However, the suitability of these systems depends on local and climatic conditions.

In CEA, greenhouses vary in design and construction materials, including glass, rigid plastic panels of polycarbonate and acrylic, and plastic-covered structures (Syed & Hachem, 2019; Vanthoor et al., 2011), where glass and rigid plastic have longer lifespans, are technologically advanced, and are born on a sturdy frame of galvanized steel or aluminum. In contrast, many plastic-covered greenhouses have a frame of PVC pipes or wood, if not aluminum or galvanized steel (Vanthoor et al., 2011).

A major issue with greenhouses is higher energy consumption through heating, cooling, and artificial lighting, which leads to more emissions of greenhouse gases (Muñoz-Liesa et al., 2022). A greenhouse with well-designed glazing materials optimizes sunlight diffusion and/or transmission of specific wavelengths of light to avoid overheating (Graamans et al., 2018; Sethi & Sharma, 2008). Additionally, greenhouse gas emissions drive innovations in energy-saving techniques, renewable energy systems (solar, wind), carbon capture, IoT (Internet of Things), AI (artificial intelligence), smart glass, and thermal energy storage (Maraveas et al., 2023). For instance, integrating sensors, AI, and cloud connectivity can be used to optimize delivery of nutrients to optimize crop performance while reducing costs (Cafuta et al., 2021).

1.3.2 Growth Chambers

Plant growth or environmental chambers create fully controlled indoor environments for the optimization of plant growth through control of light, temperature and humidity. They are frequently used in plant breeding and plant physiology research (Conn et al., 2013; Holley et al., 2022; Kang et al., 2023; Malambane et al., 2021; Yoshimatsu, 2012).

Growth chambers are available in different models, such as reach-in chambers appropriate for plant pathology and seedling growth, and walk-in chambers for applications in biotechnology and crop studies (Convion, 2025). These research tools are widely used by research institutions, universities, and agricultural and biotechnology companies studies (Convion, 2025). However, their high cost limits the scope and viability of projects for which they can be used (Katagiri et al., 2015). IoT-based systems (Chong et al., 2023), programmable systems (Padmanabha & Streif, 2019) and automated systems like RaspberryPi-Arduino (Arunachalam & Andreasson, 2022) provide innovative, low-cost solutions to increase accessibility. More advanced Enviratron systems integrate robotics with sensors to make research time- and cost-efficient with high precision (Bao et al., 2019).

1.3.3 Vertical Farming

Vertical farming (VF) is another innovative agricultural approach involving the cultivation of crops in controlled indoor environments where temperature, light, and nutrients are precisely managed. Plants are typically grown in stacked layers, sometimes across multiple stories, using hydroponics, aquaponics (which integrates hydroponics with fish farming), and aeroponics (where roots are suspended in air and misted with nutrients through emitters) (Birkby, 2016).

Mainly, VF can be divided into two groups: stacked horizontal systems and vertical growth surfaces. A stacked horizontal system consists of multiple levels of a horizontal growing system stacked on each other, maximizing the use of vertical space while maintaining traditional horizontal plant growth. It is popular for commercial purposes for growing leafy greens, strawberries, and herbs (Beacham et al., 2019). In contrast, vertical growth surfaces involve cultivating plants on vertically oriented structures, such as wall-mounted panels, cylindrical towers, columns, or hanging fabric strips. In these systems, plants may grow outward from the surface using hydroponic or aeroponic techniques, allowing for even more compact space utilization and potentially greater light exposure per plant (Lombardi & Jr, 2022). VF can be further categorized into three types based on their location and intended use: vertical home farms designed for personal use in residential settings (e.g., Agrilution, Fibonacci, Urban Cultivator), in-store vertical farms installed inside restaurants and supermarkets (e.g., Infarm), and indoor vertical factory farms, large-scale systems dedicated to commercial production in standalone buildings (e.g., AeroFarms and GrowUp Urban Farms) primarily producing crops like leafy greens, microgreens, and herbs (Jürkenbeck et al., 2019; Semananda et al., 2018)

1.3.4 Other CEA Facilities

Plant Factory with Artificial Lighting (PFAL), also known as closed plant production systems, are advanced indoor farming systems that utilize artificial lighting and controlled environments to optimize crop yield. Examples include container farms, warehouses, and vertical farms for the production of leafy greens, herbs, and transplants in Asia, North America, and Europe (Kozai et al., 2019). Among these, container farms are self-contained growing units built into repurposed shipping containers. They have become increasingly popular due to their mobility, modular design, and suitability for urban and remote locations (Beacham et

al., 2019; X. Liu, 2014). Container farms allow for year-round production with minimal land requirements, making them appealing for small-scale entrepreneurs and local food systems. However, significant costs of plant factories are electricity (28%) and labor (26%) (Liu & Teng, 2017) which can limit their viability.

Smart plant growth tents are low-cost growth chambers that have been adopted by the commercial sector. In particular, microgreens, leafy greens, and cannabis are produced in indoor tents in which light is provided entirely from supplemental light (Vee, 2024). Additionally, they enable growers to customize temperature and humidity. Commercially available brands include VIVOSUN, AC Infinity and Mars Hydro.

1.4 Brief Overview of Hydroponic Growing Systems

Hydroponic systems are generally divided into open and closed systems, also known as linear and recirculating systems, respectively. In an open hydroponic system, the nutrient solution (NS) is not reused once it is delivered to the roots, while in a closed hydroponic system, surplus solution is recirculated, replenished, and recycled (Shrestha & Dunn, 2017). Rodríguez-Jurado et al. (2020) reported that water use efficiency was higher by 55.7% in the closed system with crops such as lettuce, tomatoes, and cucumbers compared to an open hydroponic system. Similarly, nutrient use is efficient in a closed system, with the system producing 13.5 kg more fruits per cubic meter of water than an open system (Rodríguez-Jurado et al., 2020). However, closed systems have disadvantages, such as ion accumulation challenges, frequent monitoring of the NS, and additional infrastructure such as a reservoir and a pumping system (Christie, 2014). In contrast, in open systems, high water consumption and nutrient waste occur because of nutrient runoff and drainage, which can negatively impact environmental sustainability (Fathidarehnejeh et al., 2024).

Nutrient Film Technique (NFT) is a dominant closed hydroponic system wherein plant roots come in contact with a thin, continuous flow of NS inside troughs or channels, while adequate aeration of the root system occurs due to the high surface area-to-volume ratio and NS recirculation by a pump. NFT is widely used in commercial food and medicinal plant production because of easy maintenance, comfortable working height, and faster and optimal plant growth (Agustian et al., 2022; Mohammed, 2018; Walters, 2015). NFT systems save 70 to 90% of water (Sharma et al., 2019) because unused NS after passes are recycled (Rajaseger et al., 2023). NFT-based hydroponic systems have shown promise to offer better energy-use efficiency for producing leafy greens in indoor plant factories compared to deep water culture (DWC) (Gillani et al., 2023). NFT is also a valuable research tool for studying plant responses to environmental factors, including salinity, drought, and heavy metal stress, and their interactions with microbes (Atherton & Li, 2023). However, NFT requires water pumps to operate the system and depends on constant electricity (Sahat Tua Manalu et al., 2023).

In addition, other hydroponic systems include DWC, drip systems, wick systems, and aeroponic systems. In DWC systems, plant roots are submerged in a nutrient-rich solution with continuous aeration at the root level, which supports higher yields. Additionally, DWC depends on air stones to maintain adequate oxygen levels (Riggio et al., 2019). However, sometimes the roots of the plant will not be submerged if the NS level is insufficient, resulting in plant death (Nursyahid et al., 2021). Drip systems drip nutrients gradually through emitters, thus ensuring water efficiency, but demand regular pH monitoring for optimal growth (Grigas et al., 2019). Wick systems use capillary action to supply nutrients, making them simple and low maintenance, but they are limited by their capacity for large-scale or long-term plant growth (Semananda et al., 2018). Aeroponics, which maximizes root aeration, offers precise nutrient delivery but results in

challenges with limited nutrient and water availability for plant growth to the roots and the possibility of pump failure (Li et al., 2018).

1.5 Nutrient Management in Hydroponics

1.5.1 Overview of Essential Nutrients

There are 17 essential elements required by plants, necessary for proper growth and development. Non-fertilizer nutrients are carbon (C), hydrogen (H), and oxygen (O), which are obtained through air and water (Asao, 2012). Macronutrients include nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) that are required in higher quantities, while micronutrients of iron (Fe), manganese (Mn), zinc (Zn), boron (B), molybdenum (Mo), chlorine (Cl), copper (Cu) and nickel (Ni) are required in lower quantities (Sathyanarayan et al., 2023). The role of nutrients, along with the characteristics of their deficiencies and excesses, is summarized in Table 1.1.

Table 1.1. Summary of nutrient functions, deficiency symptoms, and toxicity effects (Kamboj et al., 2018; Khan et al., 2018; Resh, 2022a; Roychoudhury & Chakraborty, 2022; Tsukagoshi & Shinohara, 2020)

Nutrients	Functions in Plants	Deficiency Symptoms	Excess Effects
Non-fertilizer nutrients			
H	Water formation		
O	Release of energy from sugar		
C	Formation of organic compounds		
Macronutrients			
N	Chlorophyll, amino acids, and protein synthesis	Lower leaf yellowing, whole leaf color fading, and a reduction in plant vigor	Chlorosis (ammonium N), luxuriant growth, and succulent growth (due to nitrate N)
P	Constituents of cell membranes, enzymes, ATP, Photosynthesis and growth	Growth inhibition, anthocyanin pigmentation, and the dark greening of lower	Rare cases. Cucumber with white spots

K	Enzyme activity	leaves and the lowest portion of the stem Brown patches on leaves, yellowing of the lower leaf margin or interveinal portion, and outward curling of the leaves	Rare. Cause of Ca, Mg deficiency
Ca	Cell growth	Tip-burn, blossom-end rot, root rot	Rare cases. Causes of deficiencies in K and Mg. Raising pH of NS, which results in a micronutrient deficit
S	Formation of amino acids and proteins	Lower leaf color fading or yellowing	Rare cases. Cause of K, Ca-deficiency
Mg	Enzyme activation	Yellowing of the interveinal part or the edge of the lower leaves	Rare cases. Cause of K, Ca deficiency
Micronutrients			
Fe	Used in photosynthesis	Chelate Fe is rapidly absorbed by fresh leaves when they are moved from soil to soilless conditions, causing dark spots. Ozone or UV radiation sterilizing a nutritional solution may cause Fe to precipitate.	Yellowing of the root or chlorosis of the interveinal portion of young leaves
B	Vital for reproduction	Fruit drop, lateral root poor growth, stem and leaf hardness, and new leaf deformity	Lower leaf curling, lower leaf browning with dark patches, and lower leaf yellowing at the margins
Cl	Helps root growth	Mortality and inhibition of shoot tip growth	Root death and fibrous tubes (quality decline)
Cu	Enzyme activation	Leaf tip wilting or necrosis, defoliation	Upper leaf yellowing, root browning, and inadequate lateral root growth
Mn	Components of chlorophyll	Brown patches and a yellowing or dirty light green interveinal portion of the middle or higher leaves	Browning of lower leaf veins and discoloration of the roots
Zn	Components of enzymes	Yellowing of the interveinal portion of	Top leaf yellowing and root inhibition.

		leaves, rosette leaves, yellow-brown patches, and inhibition of leaf growth	Reasons for the Fe deficiency
Mo	N Fixation	Leaves malformation, yellowing, or necrosis of the interveinal part of leaves	Yellowing of lower leaves, red-purple coloring of veins.
Ni	Constitutes of Urease, N metabolism, and biological N fixation, C	Marginal chlorosis of leaves, premature senescence, poor germination, poor seed set, sometimes plant necrosis, Deficiency related to deficiency of copper and zinc fertilizers	Inhibition of plant development (shoots and roots), caused by Zn or Fe deficiency, which results in leaf chlorosis, poor branching, deformed plant parts, decreased yield, leaf spotting, abnormality in flower shape, mitotic root tip disturbance

1.5.2 Nutrient Solution Formulation

1.5.2.1 Principles of Nutrient Solution Formulation in Hydroponics

The nutrient solution (NS) in soilless systems must meet the following criteria: (a) provide all the essential plant nutrients; (b) all elements should be in ionic forms; (c) optimal range of the ion and total ion concentration for plants; (d) free from toxic substances and pathogens; (e) the pH should remain stable around 5.5-6.5; (f) it is ideally made up of relatively inexpensive fertilizers; (g) ion concentration, ion ratio, and pH should remain stable during cultivation, especially in DWC and NFT; and (h) the solution must provide adequate dissolved oxygen for root respiration (Tsukagoshi & Shinohara, 2020).

1.5.2.2 Classical Nutrient Solution Formulations for Hydroponic Systems

There are various standard NS formulations suggested by different scientists, which are listed below:

Table 1.2. General nutrient solution (NS) formulation for hydroponic crop production in mg·L⁻¹

(Cooper, 1988; Hewitt, 1966; Hoagland & Arnon, 1938; Smith et al., 1983; Steiner, 1984; Stoughton, 1969)

Element	Hoagland and Arnon (1938)	Steiner (1984)	Hewitt (1966)	Copper (1988)	Stoughton (1969)	Ruakura (1983)
N	210	168	168	200–236	150	264
P	31	31	41	60	35-70	40
K	234	273	156	300	200-250	238
Ca	160	180	160	170-185	300	127
Mg	34	48	36	50	50	21
S	64	336	48	68	-	60
Fe	2.5	2-4	2.8	12	5	3
Mn	0.5	0.62	0.54	2	0.1	0.5
B	0.5	0.14	0.54	0.3	0.2	0.5
Zn	0.05	0.11	0.065	0.1	1	0.25
Cu	0.02	0.02	0.064	0.1	0.5	0.04
Mo	0.01	-	0.04	0.2	0.05	0.01
Na		-		-	-	15

1.5.3 Comparative Overview of Commercial Fertilizer Formulations

Formulation of NS as shown in Table 1.2 can be difficult for small hydroponic growers to manage (Mattson & Peters, 2014). Consequently, many producers would rather utilize commercially available water-soluble fertilizers, which are easier to handle and provide standardized nutrient compositions (Mattson & Peters, 2014). For example, commercial NSs such as Jack's Hydro FeED 5-12-26 combined with Calcium Nitrate 15.5-0-0 have been shown to support comparable or improved plant growth compared to traditional formulations like Hoagland or Steiner, particularly in leafy greens production (Mattson & Peters, 2014). Similarly, Peters Professional (e.g., Peters Excel 15-5-30) has been reported to provide more stable nutrient delivery and enhanced plant response in controlled environment settings (Bugbee, 2004; Mattson & Peters, 2014).

In parallel, interest in organic hydroponics production has grown in response to rising consumer demand for organically produced crops (Burnett et al., 2016). Organic fertilizers are

derived from naturally occurring materials of biological or mineral origin and have the potential to reduce nitrate concentration in crop species. However, there are some challenges with organic fertilizers in recirculating hydroponic production, such as lower yield, pH fluctuations, limited pH adjustment options, unreliable EC monitoring, and nutrient imbalances (Williams & Nelson, 2016).

1.5.3.1 Practical Considerations and Trends in Nutrient Source Selection

Due to the shortcomings of earlier standard formulations, various commercial fertilizers are offered in one-, two-, and three-bag forms. Three-bag fertilizers are particularly used for fruiting crops such as tomatoes and cucumbers. The benefits of these commercial fertilizers are numerous. The whole nutritional profile required for hydroponic plant growth is supplied by commercial fertilizers, which include micronutrients like Fe and Zn as well as macronutrients like N, P, and K. Balanced composition provides plants with all essential nutrients required for healthy growth and development. With detailed mixing and application directions, these fertilizers are simple to use. Commercial fertilizers are easily accessible and have specialized formulations. Downsides of commercial fertilizers may be nutrient waste and accumulation of harmful compounds such as nitrates (Phibunwatthanawong & Riddech, 2019; Saputri et al., 2021; Umar & Iqbal, 2007).

1.5.3.1.1 One-part Fertilizers

One-part fertilizers are commonly used in a variety of hydroponic, aquaponic, and aeroponic systems. They are designed to provide an ideal ratio of all essential nutrients, encouraging robust plant development and yields. For gardeners looking for a straightforward yet efficient solution for leafy greens and other crops, these products are a reliable option. Their composition includes a balanced combination of micronutrients, including vital iron chelates

like DPTA, EDDHA, and EDTA. Water with an alkalinity of 40–200 ppm is ideal for their use (Table 1.3; JacksNutrients, 2025; Mattson & Peters, 2014).

There are also very effective liquid fertilizer solutions made specifically for crops that are cultivated hydroponically. Important macro and micronutrients are included in this one-part formula. Organic C sources, amino acids, aquatic plant extracts, and beneficial microbes are also included, promoting healthy plant growth (Table 1.3; TPS, 2022).

Table 1.3. Composition of nutrients and their concentration in one-part fertilizer of Johnny’s nutrients, TPS One™, Aerogarden, Jack’s J.R. Peters, and Dyna Grow liquid plant food for leafy green vegetables

Composition	Jack's Nutrients	TPS One™	Dyna Gro
Total N	16%	3.20%	7%
Available Phosphoric acid (P ₂ O ₅)	4%	6.50%	3.9%
Soluble Potash (K ₂ O)	17%	4.10%	4.1%
Mg	1.50%	0.50%	0.35%
S	-	0.04%	-
B	0.02%	0.002%	0.01%
Cu	0.01	0.002%	0.03%
Fe	0.22%	0.01%	0.07%
Mn	0.05%	0.007%	0.03%
Mo	0.01%	-	0.01%
Zn	0.05%	0.004%	0.01%
Ca	4%	1%	1.39%

However, these fertilizers are often too low in S, an essential element in plant growth and metabolism (Kong et al., 2024). Studies have demonstrated various improvements in the growth, yield, and quality of plants after supplementing sulfur with one-part fertilizers. For instance, supplementing at least 10 mg·L⁻¹ of sulfur increases shoot biomass, photosynthesis, and leaf chlorophyll content and hence assures optimum performance of hydroponically grown lettuce with low sulfur levels either in the base fertilizer or water source (Kong et al., 2024).

1.5.3.1.2 Two-part Fertilizers

There are various two-part commercial-type fertilizers such as Crop King’s, Johnny’s nutrients, Peter's Professional nutrients, and Optimum Grow (Table 1.4).

Table 1.4. Composition of nutrients and their concentration in two-part fertilizer of Crop King and Johnny's nutrients, and Peters Professional for leafy green vegetables

Composition	Crop King's	Jack's Nutrients	Peter's Professional Nutrients	Optimum Grow	Jack's, J.R. Peters	Jack's, J.R. Peter's
Part A						
Total N	4.30%	5%	5%	37.62%	5%	5%
Available Phosphoric acid (P ₂ O ₅)	9.30%	12%	11%	-	4.8%	5.2%
Soluble Potash (K ₂ O)	35%	26%	25%	25.55%	21.6%	21.6%
Ca	-	-	-	36.82%	-	-
Mg	3.90%	6.30%	6%	-	1.23%	0.81%
S		8.50%	8%	-	-	-
B	0.02%	0.05%	0.05%	-	0.01%	0.01%
Cu	0.01%	0.02%	0.02%	-	0.01%	0.01%
Fe	0.21%	0.30%	0.30%	-	0.06%	0.08%
Mn	0.01%	0.05%	0.05%	-	0.01%	0.01%
Mo	0.01%	0.02%	0.01%	-	0.01%	0.01%
Zn	0.02%	0.02%	0.02%	-	0.01%	0.01%
Part B						
Total N	15%	15%	15%	12.76%	-	15.5%
P	-	-	-	11.37%	-	-
K	-	-	-	46.50%	-	-
Ca	19%	18%	18%	-	~19%	~15%
Mg	-	-	-	13.07%	-	-
S	-	-	-	16.30%	-	-

The major benefit of using two-part fertilizers is to prevent the precipitation of Ca and P in concentrated stock solutions. Impact of two-part and one-part fertilizers on pepper and eggplant was observed by Singh et al. (2019). The two-part fertilizer included 5N-4.8P-21.6K (Jack's J.R. Peters) or 5N-5.2P-21.6K (Jack's J.R. Peters), while the one-part fertilizer included 7N-3.9P-4.1K (Dyna Gro). The two-part fertilizers (5N-4.8P-21.6K or 5N-5.2P-21.6K) resulted in higher sweet pepper growth (Singh et al., 2019). Their findings for eggplant, however, revealed a great deal of variation, most likely due to nutrient accumulation in the small hydroponic system. Their results highlight the need for fertilizer management strategies focusing on the specific nutrient needs of diverse crops to optimize growth in hydroponic systems.

1.5.3.1.3 Three-part Fertilizers

Three-part systems go a step further in nutrient management by segregating nutrients into three separate formulations, generally focused on macronutrients, secondary nutrients, and micronutrients. This can allow for greater customization and precision in the application of nutrients. Research has shown that three-part systems have optimized nutrient uptake and improved overall plant health, especially in high-value fruiting crops such as strawberries and tomatoes. HortAmerica formulated the fertilizer (9N-7P-37K), which needs to be supplemented with calcium nitrate and magnesium sulfate for growing lettuce, strawberries, and tomatoes. Similarly, CropKing formulated three-bag fertilizer for growing tomatoes. However, it must be prepared in three separate stock solutions (Table 1.5).

Table 1.5. Composition of nutrients and their concentration in the three-part fertilizer for crop (Hughes, 2019)

Composition	Hort America	Crop King
Part A		
N	9%	4.40%
P	7%	13%
K	37%	34%
Mg	-	3.7%
B	0.09%	275 mg·kg ⁻¹
Cu	0.05%	95 mg·kg ⁻¹
Fe	0.50%	1000 mg·kg ⁻¹
Mn	0.19%	800 mg·kg ⁻¹
Mo	0.01%	40 mg·kg ⁻¹
Zn	0.19%	270 mg·kg ⁻¹
Part B		
N	15.5%	15.5%
Ca	26.3%	19%
Part C		
N	-	13.5%
K	-	46.2%
Mg	9.6%	13.5%
S	13%	46.2%

Thus, commercial fertilizers are available in various forms, with two-part being the most preferred for leafy greens in hydroponic production (Mattson & Peters, 2014). In contrast, one-part fertilizers are convenient and popular for beginners but lack adequate S and Ca and require frequent supplementation, while three-part fertilizers are mostly used for high-value fruiting crops such as tomatoes and strawberries but require more complex preparation and additional supplementation.

1.5.4 Nutrient Management Overview

Different strategies have been adopted for nutrient management in a recirculating hydroponics system. One approach is the mass balance approach, which accounts for nutrient input, plant uptake, and nutrient retention within NS (Bugbee, 2004; Langenfeld et al., 2022). Despite the advantages of using a mass balance approach, nutrient replenishment strategies should be easy to manage and maintain over time. According to Houston et al. (2023), replenishment solutions can stabilize fluctuations within the root zone; however, imbalances still occur with both standard replenishment and species-specific NS replenishment. Additionally, EC-based nutrient management is frequently utilized in recirculating systems (Miller et al., 2020; Resh, 2022; Savvas & Gruda, 2018; Walters et al., 2020).

Among current practices, EC-based nutrient management in recirculating hydroponic systems is popular because of its fast measurement, simplicity, and low cost (Fathidarehnejeh et al., 2024). EC measures the total dissolved ion that allows the growers to know the overall strength of nutrients in the solution, which is also adopted as a standard method by commercial growers (Samarakoon et al., 2020). Thus, EC measurement is considered a standard indicator of hydroponic fertilizer concentration (Ahn et al., 2021; Lu et al., 2017; Signore et al., 2016).

However, it represents the total dissolved ion available in the solution without the measurement of individual ion concentration and causes an imbalance over time (Steidle Neto et al., 2014).

1.6 Nutrient Uptake

Plants absorb nutrients through two primary means: apoplastic transport via interconnected cell walls and symplastic transport via cellular ion channels. Apoplastic pathways enable plants to absorb ions selectively without using energy (Tsukagoshi & Shinohara, 2020). Nutrients are divided into three categories according to the types of uptake: active, intermediate, and passive (Mitra, 2017). Active nutrients--N, P, K, and Mn--are rapidly absorbed by plants and can be extracted by roots from NS in a few hours. Passive nutrients, which include Ca, are transported through plants by mass flow and ion diffusion. Additionally, because plant transpiration is directly linked to passive nutrients, they emphasize the significance of environmental factors, including humidity, light intensity, and airflow (Mitra, 2017). The absorption rates of intermediate-uptake nutrients are lower than those of actively absorbed nutrients, but are faster than water (Mitra, 2017). Therefore, understanding the nutrient absorption rate is essential for evaluating the nutrient concentration in hydroponic or soil-based production systems (Bugbee, 2004).

1.6.1 Current Nitrogen Management Practices

Nitrogen is a critical nutrient for plants and is required in greater quantities than any other fertilizer element due to its integral role in plant growth and metabolism. As a key component of proteins, N is directly linked to vital physiological and biochemical functions that drive healthy plant growth and development, ultimately enhancing both yield and quality (Leghari et al., 2016; Subedi et al., 2023). Leaf N typically ranges from 1 to 6% of dry weight, depending on plant species, stage of maturity, and several other factors (Bryson & Mills, 2014).

Several studies have demonstrated that the form of N (nitrate or ammonium) has a greater impact than the total amount of N on crop yield and marketable quality of vegetable species (Chatzigianni et al., 2018; Perner et al., 2011). Nitrate uptake and assimilation are energy-intensive because of the need for assimilation into ammonium via enzymes nitrate reductase and nitrite reductase (Milton & Minter, 2017). Ammonium can be directly assimilated into organic N and easily absorbed by plants; ammonium toxicity can be a problem at high concentrations (Valkama et al., 2013).

As examples, high ratios of nitrate promote growth and mineral accumulation, while ammonium suppresses growth and photosynthesis for watermelon seedlings (Na et al., 2014). In basil, nitrate supports growth, while ammonium enhances polyphenolic acids in green basil and anthocyanins in purple basil (Prinsi et al., 2020). Similarly, the N balance is also important in systems like hydroponics, where the growth, nutrient uptake, and abiotic stress tolerance depend on the ratio of ammonium to total N feeding (Bensalem et al., 2020; Chrysargyris et al., 2021). Studies also show that N form impacts nutrient composition and stress responses, such as reducing Na accumulation, and enhances antioxidant activity in NaCl-stressed plants (Bensalem et al., 2020; Xiang et al., 2020). In hydroponics, ammonium to nitrate ratios of the 0:100 ratio resulted in better performance for chives while a ratio of 20:80 resulted in better performance for basil (González-García et al., 2009). In aeroponic lettuce production, NS containing 86.8% nitrate and 13.2% ammonium achieved the highest average weight of 743 g per plant of lettuce (Demšar & Osvald, 2003). Additionally, ammonium to nitrate ratios of 25:75 increased the plant height, leaf number, and biomass in hydroponically grown Chinese cabbage (Song et al., 2011).

Excess ammonium is toxic to plants at high concentrations, causing oxidative stress, stunted growth, chlorosis, and nutrient deficiencies (Bryson & Mills, 2014). While ammonium

can enhance foliar greening, it starves plants of carbon resources that are needed for growth and can harm roots; Si supplementation has been observed to reduce ammonium toxicity (Barreto et al., 2016; Campos et al., 2016). In contrast, nitrate represents a major source of N in most crops but can accumulate excessively in plants, especially leafy vegetables, depending on environmental conditions like temperature and CO₂ (Colla et al., 2018). Excessive nitrate accumulation in vegetables is a health concern, as it may contribute to conditions such as methemoglobinemia and has been linked to potential long-term health risks (Colla et al., 2018).. Environmental factors (temperature, light, and CO₂) and fertilizer management can play a crucial role in the nitrate accumulation in some vegetable species (Bian et al., 2020). For instance, low light intensity increased the nitrate accumulation in greenhouse-grown lettuce because of lower nitrate reductase (NR) activity (Gaudreau et al., 1995). However, Parks et al., (2008) highlight that N form plays a key role, and to a lesser extent, light levels. High light intensity (220 $\mu\text{mol}\cdot\text{mol}^{-1}$) and low N (7 $\text{mmol}\cdot\text{L}^{-1}$) decreased the nitrate concentration in lettuce (Fu et al., 2017). Furthermore, temperature also plays a role in nitrate accumulation, with high temperatures during the summer increasing accumulation of nitrate in lettuce (Fallovio et al., 2009).

Molybdenum plays a role in nitrate reductase and its deficiency can lead to increased nitrate accumulation in plant tissue. Addition of micronutrients such as Mo (0.5, 1.5, and 3.0 $\mu\text{mol}\cdot\text{L}^{-1}$) to NS decreased nitrate concentration in hydroponically grown lettuce, escarole, and curly endive (Moncada et al., 2018). Similarly, Fe has a role in nitrate reductase (Colla et al., 2018). A previous study by Borlotti et al. (2012) reported that Fe deficiency in NS limited the nitrate reductase activity and downregulated the nitrate reductase transcription in the leaves of cucumber. Further strategies, such as pre-harvest nitrate deprivation, have been studied to reduce

the accumulation of nitrate and enhance the nutritional quality of fresh produce (Borgognone et al., 2016; Ciriello et al., 2021). Consideration of the environment, cultivation practices, cultivar, and fertilizer management are necessary to produce vegetable species with lower nitrate.

1.6.2 Current Phosphorus Management Practices

Phosphorus (P) plays a crucial role in protein activation, energy transmission, respiration, photosynthesis, and metabolic control. In general, vegetative crops have a total P content of 0.1% to 0.5% (He et al., 2006), while in mature leaves, it falls to 0.2% and 0.5% (Bryson & Mills, 2014). Early crop growth is greatly aided by P; higher P levels at the beginning of the growing season led to greater shoot length, leaf area, and shoot and root weight (Abbasian et al., 2018). Hydroponic systems for more efficient application of P, reducing waste while maintaining yield. As an example, basil and parsley show no deficiency symptoms at 5–10 mg·L⁻¹ P in hydroponics (Currey et al., 2020). Similarly, basil can be grown hydroponically with high NaCl (salinity or brackish water) and low P concentrations without significantly affecting production or quality, indicating that salt and P levels can be controlled separately for greater environmental and economic sustainability (Germano et al., 2022).

1.6.3 Current Potassium Management Practices

Potassium (K) is the second most important mineral nutrient for plants after N. It is essential for maintaining physiological functions like photosynthesis, protein synthesis, and enzyme activity, as well as vegetative development (Kanai et al., 2011). Its demand is highest before flowering and during rapid vegetative growth, and it is strongly correlated with fruit quality and yield. In many plants, a K deficit in the late vegetative or reproductive phases results in reductions in quality (Ernest., 2023).

Potassium absorption is adversely affected by high salinity because of competition with Na for absorption sites, which leads to stunted plant growth and exacerbation of other stressors (Marques et al., 2018). Potassium chloride (KCl) overuse can also be harmful since extended exposure can raise plant chloride levels, which can cause necrosis, chlorosis, and decreased yield. Excessive amounts of chloride are detrimental, even though they are required for functions like water photolysis and electron transport in photosynthesis (Khasawneh, 1971).

Potassium is also necessary for human health; however, excessive intake of this element can cause hyperkalemia, which can result in deadly cardiac arrhythmia, especially in people with compromised kidney function. On the other hand, as a less frequent condition, K depletion can cause cramps, muscle weakness, and irregular heartbeats (Kovesdy et al., 2017; McDonough & Youn, 2017). In hydroponics, crop productivity and K levels for human nutrition can be balanced using quantitative K control. Reducing the K supply in hydroponic systems reduced cherry tomato fruit yield, K content, and acidity (Tsukagoshi et al., 2021). Their 1/4 K (1.8 g per plant) and 1/8 K (0.9 g per plant) treatments produced cherry tomato fruit K levels below 100 mg·100 g⁻¹, which qualifies them for low-K dietary needs. This emphasizes the necessity of specialized K management techniques to solve environmental issues in agricultural systems and maximize the health of plants and people.

1.7 Postharvest quality

1.7.1 Appearance

Appearance is the most important quality factor regarding product commercialization (Brasil & Siddiqui, 2018). It includes color, shape, size and gloss which are the foremost criteria the influence consumer preferences and so are used to evaluate the immediate quality of fresh vegetables and fruits (Clydesdale, 1991).

1.7.2 Texture

Texture represents firmness, crispness, juiciness, mealiness, and toughness, depending on the crop. It is influenced affected by traits such as cellular organelles and biochemical constituents, water content or turgor, and cell wall composition (Sams, 1999).

1.7.3 Flavor

Flavor is an essential aspect of product quality that includes sweetness, sourness (acidity), bitterness, aroma, and off-flavors (Brasil & Siddiqui, 2018). Flavor quality involves perception of the tastes and aromas of many compounds.

1.7.4 Nutritional quality

Nutritional quality is one of the quality factors considered in the supply chain of vegetables and fruits. Generally, vegetables are considered “protective foods” (Natesh et al., 2017). Fresh vegetables and fruits play a significant role in human nutrition as they are rich in vitamins (A, B, thiamine and niacin), minerals, dietary fiber, flavonoids, carotenoids, phytochemicals and antioxidants (Gibson & Hotz, 2001). The nutritional quality of different leafy green species is presented in Table 1.6.

Table 1.6. Nutritional quality of leafy green vegetable species

Species	Nutritional quality	References
Lettuce	Low in calories, fat and sodium, good source of fiber, iron, folate, vitamin C and bioactive compounds. Red lettuce contains higher phenolic compounds than green lettuce.	Conversa et al. (2021); Kim et al. (2016); Lei & Engeseth (2021)
Arugula	Have pungent and bitter flavor due to accumulation of glucosinolates and antioxidants and also rich in P, S, Fe, vitamin A and C	Freitas et al. (2017); Morais et al. (2020)
Kale	Source of fiber, minerals (K and high Ca), prebiotic carbohydrates, unsaturated fatty acids and different vitamin (A and C) antioxidants, glucosinolates, and flavonoids	Satheesh & and Workneh Fanta (2020)
Basil	Rich in essential oils and compounds such as polyphenols, phenolics, and flavonoids and used for seasoning	Shahrajabian et al. (2020).

Malabar	Rich in vitamin A and C, carotenoids, phenols, alkaloids, tannins content, bioflavonoid, organic acid, betacyanin, and minerals such as Ca, P, K, Mg	Chaurasiya et al. (2021); Qiu & Liu (2020)
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1.8 Electrical Conductivity in Hydroponic Systems

Monitoring EC in a hydroponic system is important to prevent two primary risks: the NS from getting too concentrated, which leads to salt stress, or too diluted, which may result in nutrient deficiencies. EC is crucial because it directly impacts the development, yield, and nutritional value of plants in hydroponic systems (Tzortzakis et al., 2020).

1.8.1 Current Status of EC Optimization

There has been extensive research on EC optimization for lettuce. However, limited attention has been given to other leafy greens such as kale, arugula, and Swiss chard, which are commercially important for hydroponic production.

For kale, limited research has been conducted on EC optimization (Yang et al., 2024). Other studies with kale focused on factors including NS depth and cooling effects (Catigday et al., 2023; Tan et al., 2023), N and Mg effects, KNO₃ concentration, salinity stress, and calcium nitrate (Mao et al., 2022; Melo et al., 2024; Oliveira et al., 2023). Additionally, environmental conditions such as temperature, humidity, CO₂, light intensity, and salinity have also been explored (Chowdhury et al., 2021; Pascual et al., 2024).

Research on EC optimization in arugula is also limited. Yang et al. (2021) reported an ideal EC range of 1.0-1.8 mS·cm⁻¹ for arugula ‘Standard’, while other studies have explored the inoculation of beneficial microbes to boost production and crop nutrition (Gato et al., 2023) or NS replenishment (Houston et al., 2023) for this crop.

In Swiss chard, most studies have focused on the performance of different water-soluble fertilizers on cultivars (Singh et al., 2019); the ratio of effluent to inorganic fertilizer in aquaponic

systems (Abdelfattah, 2023); harvest time and N dose (Vanegas et al., 2016); and plant spacing, frequency of harvesting, and growing conditions (Bulgari et al., 2017; Maboko et al., 2009).

In summary, profound information gaps exist in the optimization of EC for less-studied leafy greens such as arugula, kale, Swiss chard, and Malabar spinach, whereas crops like lettuce have been thoroughly evaluated in hydroponic production systems. This emphasizes the need for further research regarding the EC optimization to enhance the growth, production, and nutritional quality of these less-studied crop species.

1.8.2 EC and Nutritional Quality Optimization Based on Species and Season

The optimum EC range for different crop species is shown in Table 1.7. This EC range can vary based on the cultivar and environmental conditions.

1.8.2.1 Lettuce

An optimum EC, such as $1.8 \text{ mS} \cdot \text{cm}^{-1}$ for lettuce ‘Salanova Red Butter’ and ‘Salanova Green Butter’, will enhance crop yield, while EC higher than the optimum will result in decreased yield, physiological disorders (e.g., tipburn), and variable responses according to environmental conditions and cultivars (Samarakoon et al., 2019). However, lettuce ‘Batavia-Caipira’ displayed higher growth (fresh weight, dry weight, number of leaves, and leaf area) at EC 1.2 and $0.9 \text{ dS} \cdot \text{m}^{-1}$ compared to EC 0.5, 0.7 and $2 \text{ dS} \cdot \text{m}^{-1}$ in vertical farming (Hosseini et al., 2021). Additionally, in the case of green-leaf lettuce ‘Winter Density’ and red-leaf lettuce ‘Rhazes’, NS EC levels of 1.0, 2.0, and 4.0 mS cm^{-1} significantly affected biomass, water content, and the uptake of various nutrients, with elevated EC ($\approx 4.0 \text{ mS} \cdot \text{cm}^{-1}$) optimizing yield and quality, especially during spring and fall growing seasons (Sublett et al., 2018).

A study comparing seven lettuce cultivars at low ($<1.3 \text{ dS} \cdot \text{m}^{-1}$) and high ($10 \text{ dS} \cdot \text{m}^{-1}$) EC revealed that greater EC increased the amount of chlorophyll and nitrate while decreasing fresh

weight (Kappel et al., 2021). However, lettuce ‘Limeira’ had the lowest nitrate concentration ($684 \mu\text{g}\cdot\text{g}^{-1}$ fresh weight), while ‘Cencibel’ had the highest ($4396 \mu\text{g}\cdot\text{g}^{-1}$ fresh weight) (Kappel et al., 2021). Similarly, lettuce ‘Cegolaine Bibb’ was tested with a range of EC (0.5, 1, 2, and $4 \text{ mS}\cdot\text{cm}^{-1}$) across summer, winter and spring seasons. Stomatal conductance and fluorescence decreased in summer and spring but increased in winter with higher EC ($4 \text{ mS}\cdot\text{cm}^{-1}$). Fresh weight increased with higher EC in winter and spring (Adhikari et al., 2024). Furthermore, EC $3.5 \text{ dS}\cdot\text{m}^{-1}$ improved the thickness, color, and antioxidative properties of oak-leaf lettuce in fall, but during late spring, this same variety responded better in yield and processability to EC of $2.5 \text{ dS}\cdot\text{m}^{-1}$ (Conversa et al., 2021).

Beyond yield, EC and salinity levels also affect the nutritional and quality traits in lettuce. For instance, moderate levels of salinity (200 mM NaCl) favor the biosynthesis of phenolic compounds, and more phenolics accumulated in red-leaf lettuce than in the green-leaf variety (Sgherri et al., 2017). Additionally, long-term irrigation with relatively low salt concentration (5 mM NaCl) increased carotenoid content, including lutein and β -carotene, by 37% and 80%, respectively, in romaine lettuce without affecting the color or visual quality of the plants. In contrast, short-term salt treatments resulted in a decline of the phenolic content and did not significantly affect the carotenoids (Kim et al., 2008).

1.8.2.2 Arugula

Hydroponically grown arugula ‘Standard’ showed optimal yield and quality with good nutritional value (glucosinolates and carotenoids) and lower nitrate levels when the NS was maintained at 1.5 to $1.8 \text{ dS}\cdot\text{m}^{-1}$ (Yang et al., 2021). In baby-leaf arugula, an optimum EC of $3.5 \text{ dS}\cdot\text{m}^{-1}$ optimized yield, leaf firmness, visual quality, and antioxidant compounds while minimizing nitrate accumulation (Bonasia et al., 2017).

1.8.2.3 Kale

In kale ‘Dwarf Blue Curled Scotch,’ increasing the EC from 0.5 to 2.0 dS·m⁻¹ in the NS increased plant height and leaf width, stem diameter, root volume, fresh weight, tissue N concentration, nitrates, and SPAD readings. However, Ca concentration at the petiole and leaf tissue during the experiment decreased (Martínez-Castillo et al., 2022). Additionally, the fresh and dry weight of kale ‘Red Russian’ increased linearly with increasing EC (0 to 2.1 mS·cm⁻¹) without affecting the vitamin C, anthocyanin, and phenolic compounds, while the tissue mineral nutrients (P, K, Ca, Mg, S, Fe, Mn, B, Cu, Mo, Zn in the leaf) were highest at EC 1.5 and 1.8 mS·cm⁻¹ and N and NO₃⁻¹ were highest at EC 2.1 mS·cm⁻¹ (Yang et al., 2024).

1.8.2.4 Basil

The highest shoot and leaf growth was observed in basil ‘Genovese’ at EC 3.0 dS·m⁻¹ in a hydroponic system (Ren et al., 2022). However, lower levels of 0.5 and 1.0 dS·m⁻¹ significantly enhanced the antioxidant capacity and total phenolic content of basil (Ren et al., 2022). Moreover, Walters & Currey (2018) reported that increasing the EC from 0.5 to 4.0 dS·m⁻¹ did not influence the growth and morphology in basil ‘Nufar’, ‘Lime’ and ‘Holy’. However, in the same study, N, P, K, S, Zn, B, and Cu increased with increasing EC, while Ca and Mg remained stable.

1.8.2.5 Malabar spinach

Malabar spinach, a tropical leafy vegetable, is widely cultivated in south and southeast Asia because of its nutritional and medicinal qualities (Deshmukh & Gaikwad, 2014). It has been tested and successfully grown in soil and soilless conditions with organic biogas manure in China (Liu et al., 2009). In the United States, it has been cultivated in Hawaii and Puerto Rico in a soil-based cultivation system, suggesting adaptability to tropical and subtropical environments.

Parkell et al. (2016) and Qiu & Liu (2020) provided a general overview of Malabar spinach growth techniques for Florida, highlighting the potential appeal among customers who are interested in authentic Asian cuisine.

Malabar spinach represents a potential new species for hydroponic cultivation. Previous study by Dhananjani & Pakeerathan (2023) with organic NSs such as fish emulsion with EC adjusted to 1.8 to 2.4 dS·m⁻¹ were found to be promising for its growth in hydroponics system. However, research on EC optimization using inorganic nutrient formulations remains limited.

1.8.2.6 Other Crops

In Pak choi ‘Shanghaiqing’, a medium EC of 1.8 and 2.4 dS·m⁻¹ optimized plant biomass, leaf photosynthesis, and vegetable quality (taste, leaf color, ascorbic acid content, and antioxidant enzyme activity (Ding et al., 2018). Furthermore, in hydroponically grown cilantro ‘Santo’, dill ‘Fernleaf’, and parsley ‘Giant of Italy’, different EC levels (0.5 to 4.0 dS·m⁻¹) did not result in any differences in shoot fresh and dry weight (Currey et al., 2019). However, Ca and Mg decreased with increasing EC, while K remained unaffected by EC levels (Currey et al., 2019). In fruiting crops, higher EC levels of 10 dS·m⁻¹ increased the quality of tomato fruits by improving vitamin C, lycopene, β -carotene, total soluble solids, and antioxidative capacities by up to 35%, though yield and firmness were reduced (Krauss et al., 2006). Similarly, increasing EC from 2.0 to 4.1 dS·m⁻¹ improves dry matter, carbohydrates, starch, and vitamin C content in fruits of zucchini squash, although higher salinity of 4.1 dS·m⁻¹ reduced yield (Rouphael et al., 2006).

These findings highlight the importance of optimizing EC based on the tolerance levels of each crop species and growing season to optimize crop growth and quality.

1.8.3 Effect of Sub- and Supra-EC in Hydroponic Production

Excessive EC can negatively impact plant health. High EC creates osmotic stress, which disrupts plants' water uptake and impairs physiological functions. This situation can ultimately lead to stunted growth, reduced yields, and nutrient imbalances (Chrysargyris et al., 2021; Grattan & Grieve, 1998; Juan et al., 2005). Higher EC ($2.1 \text{ mS}\cdot\text{cm}^{-1}$) can result in lower fresh weight, likely due to osmotic stress and increased nitrate accumulation in plants (Yang et al., 2021). Higher EC also affects water uptake and photosynthesis efficiency. An inflow/efflux EC of $8.4/14.3 \text{ dS}\cdot\text{m}^{-1}$ caused a 28% reduction in leaf conductance and a 29% reduction in transpiration in crisp head lettuce 'Ryder' (Costa et al., 2001). Similarly, under high salinity conditions, maximum photosynthetic rates were reduced by 49% in tomato 'Mariachi' and 47% in tomato 'Rapsodie' during vegetative growth, and leaf conductance decreased by 15% during reproductive growth (Wu & Kubota, 2008). Very high EC levels of $9.6 \text{ dS}\cdot\text{m}^{-1}$ are disadvantageous to the growth, quality, and physiological performance of Pak choi 'Shanghaiqing' (Ding et al., 2018). Moreover, high EC of $10 \text{ dS}\cdot\text{m}^{-1}$ induced increased nitrate accumulation and reduced yield in lettuce (Kappel et al., 2021). Furthermore, when the plants shifted from high EC of $2.6 \text{ dS}\cdot\text{m}^{-1}$ to low EC of $0.8 \text{ dS}\cdot\text{m}^{-1}$, they displayed higher leaf connectivity early in the cropping cycle but lost leaves towards the end of the cultivation period, possibly due to increased abscission rate of leaves (Sakamoto & Suzuki, 2020). Moreover, high EC causes salinity stress, apart from physiological stress, which impairs nutritional intake, creating osmotic imbalances. Overapplication of nutrients may lead to the pollution of the environment and waste of nutrients (Meselmani, 2022).

In contrast, low EC can cause nutrient deficiency and may affect plant health and yield severely (Asao, 2012; Pinter et al., 2003; Signore et al., 2016). A lower EC of $1.2 \text{ mS}\cdot\text{cm}^{-1}$

resulted in lower fresh weight, likely due to nutrient deficiency, and also increased nitrate accumulation in plants (Yang et al., 2021). Similarly, very low levels of (EC 0 to 0.6 dS·m⁻¹) decreased the growth, quality, and physiological performance of Pak choi ‘Shanghaiqing’ (Ding et al., 2018).

It is crucial to establish ideal EC levels that encourage plant growth and prevent negative consequences of both low and high EC levels in hydroponic systems. Therefore, determining the ideal EC levels for different crops is necessary for maximizing productivity and reducing fertilizer waste (Nguyen et al., 2021).

Table 1.7. Optimal EC range of various hydroponically suitable crops
(Sharma et al., 2019; Singh & Dunn, 2016)

Crop	EC (mS·cm ⁻¹)
Lettuce	1.2 to 1.8
Spinach	1.8 to 2.3
Basil	1.0 to 1.6
Parsley	1.8 to 2.2
Pak choi	1.5 to 2.2
Tomato	2.0 to 4.0
Cucumber	1.7 to 2.0
Strawberry	1.8 to 2.2
Banana	1.8 to 2.2
Ficus	1.6 to 2.4
Okra	2.0 to 2.4
Leek	1.4 to 1.8
Celery	1.8 to 2.4
Cabbage	2.5 to 3.0
Peppers	0.8 to 1.8
Egg Plant	2.5 to 3.5
Beans	2.0 to 4.0
Asparagus	1.4 to 1.8

Rhubarb	1.6 to 2.0
Sage	1.0 to 1.6

1.8.4 Other Factors for EC Targets in Hydroponic Production

EC optimization in hydroponic production depends on several other factors, including environmental interactions, crop growth stage, and salinity and pH management.

1.8.4.1 Environmental Interaction with EC

Temperature, pH, and light also play a crucial role in the effectiveness of EC management in a hydroponic system. Slight changes in water pH and temperature can lead to a sharp decline in crop yield in hydroponic systems; therefore, these factors should be strictly controlled together with EC (Jordan et al., 2018). Furthermore, the negative effects of salinity are increased by high light intensity in combination with low air humidity (Sonneveld & Voogt, 2009). Increased EC levels under high light intensity have been found to decrease leaf area (Schwarz et al., 2002). However, the growth and production efficiency of red leaf lettuce were optimized with an EC of $2.0 \text{ dS} \cdot \text{m}^{-1}$ and a light intensity of $180 \mu\text{mol} \cdot \text{m}^{-2} \text{ s}^{-1}$ in controlled environments (Cha et al., 2012).

1.8.4.2 Growth Stage-Specific EC Requirement

The growth stage of crops must be considered when determining optimal EC in hydroponic systems. Younger seedlings tend to be sensitive to variation in EC as well as changes in nutrient compositions that may often lead to stunted growth or nutritional deficiencies if not rightly managed (Walters & Currey, 2015). In lettuce, during germination, the recommended EC range is 0.8 to $1.2 \text{ dS} \cdot \text{m}^{-1}$, while that for the production stage is 1.2 to $1.8 \text{ dS} \cdot \text{m}^{-1}$ (Hosseini et al., 2021; Samarakoon et al., 2020; Singh et al., 2019).

In fruiting crops such as tomato, EC sensitivity varies according to the growth stage. During the vegetative stage of tomato, high EC of $8.4 \text{ dS} \cdot \text{m}^{-1}$ markedly decreased transpiration and leaf conductance by 28% and 29%, respectively (Wu & Kubota, 2008). Additionally, during the early flowering stage of tomatoes, EC at $4.5 \text{ dS} \cdot \text{m}^{-1}$ enhanced sugar and total soluble solids (TSS). However, when the same EC of $4.5 \text{ dS} \cdot \text{m}^{-1}$ was applied four weeks after anthesis, both sugar and TSS declined (Wu & Kubota, 2008).

1.8.4.3 Salinity Stress and Toxicity

High fertilizer application and increased Na^+ and Cl^- can lead to high EC and salinity problems in a hydroponic system. As salinity stress progresses, it can result in nutrient imbalances, such as Na toxicity that could inhibit Ca absorption, and lead to physiological disorders such as necrosis in peppers and blossom-end rot in tomato (Van Os et al., 2016). These problems may be prevented by using salt-resistant cultivars and adjusting the frequency of fertigation (Gorbe & Calatayud, 2010). Therefore, optimal EC levels and balanced ion ratios are necessary for improving plant health (Nguyen et al., 2021).

1.8.4.4 Influence of pH

pH plays a key role in enhancing nutrient availability in hydroponic production. The optimum pH in the root zone ranges from 5.5 to 6.5. If pH is greater than 7, it may result in P, Fe, Mn, Cu, and Zn deficiency, while pH less than 5 leads to Fe, Mn or Al toxicity (Savvas & Gruda, 2018). Additionally, too low pH with high ammonium concentrations can cause Ca deficiency (Savvas & Gruda, 2018).

Crop-specific responses to pH and EC are evident. For instance, kale and basil produced higher marketable yields with low pH (5.5 to 6) and high EC ($1 \text{ to } 3 \text{ dS} \cdot \text{m}^{-1}$) in hydroponic production, while pepper and tomato performed better in aquaponic systems with low EC (0.5 to

1 dS·m⁻¹) and high pH of 7.2, even if their yields were reduced by up to 32% when compared to hydroponic systems (Wortman, 2015)

1.8.4.5 Factors Influencing EC Dynamics in Hydroponic Systems

As outlined in Section 1.4, hydroponic systems are generally classified into open and closed based on the recirculation of NS. In an open hydroponic system, NS is not recirculated, which means NS is drained after passing through the root zone (Maboko et al., 2009). In contrast, NS in a closed hydroponic system, also known as a recirculating hydroponic system, collects and reuses the NS (Fayezizadeh et al., 2021a; Savvas & Gruda, 2018).

Nutrient recirculation affects EC dynamics in hydroponic systems. For instance, nutrient intake in a closed hydroponic system changes the ion balance; some ions, like Fe, deplete rapidly, while others, such as Ca²⁺ and Cl⁻, build up but do not reach toxic levels. In contrast, in an open hydroponic system, bicarbonates and sulfates can be accumulated, which can increase EC. These findings highlight the interactions between nutrient recirculation and ion balance (Asao, 2012).

Seasonal factors and hydroponic system design also play a role in the fluctuation of EC. A previous study on lettuce comparing summer and fall using NFT and DWC by Yang et al. (2024) reported that stable EC and pH were maintained in the fall across both NFT and DWC, while summer trials reported higher EC, particularly in DWC. Therefore, optimizing EC in a hydroponic system requires an understanding of environmental factors, hydroponic system design, substrate, water quality, crop growth stage, etc., to maximize the growth and quality.

1.9 Nutrient Solution Volume

Ideally, plants should receive an optimum amount of nutrients and water to maximize yield and quality without extreme excess, which highlights the importance of nutrient management in hydroponic production. In a closed hydroponic systems, the NS is recirculated (Rouphael & Colla, 2005a; Sanjuan-Delmás et al., 2020); these systems are commercially popular because of their higher water and nutrient use efficiency (Fayezizadeh et al., 2021; Rosa-Rodríguez et al., 2020). Recirculating hydroponic systems also support environmental sustainability by reducing the environmental impact of nutrient disposal (Valenzano et al., 2008).

No standard exists for the optimal volume of NS provided for each plant in an NFT recirculating system. The only recommendations found were mentioned in Table 1.8, which in contrast with a recommendation for commercial producers of 0.25 to 1 gallon (1 to 3.8 L) per plant provided by Virginia Cooperative Extension (Mullins, 2023), and neither is based on refereed literature. Nutrient solution (NS) reservoir tanks are often sized to accommodate more volume than is required by the number of plants in the system, and this may lead to use of greater volumes of NS than necessary.

Table 1.8. Examples of volume of nutrient solutions (NS) in recirculating hydroponic systems for commercial and home production of leafy greens

Brand or Company	Purpose	Volume of NS (gallons)	No. of plants	L per plant	References
American Hydroponics	Commercial production	1800	3825	1.7	(AmHydro, 2025)
Crop King	Research/educational	5	40 - 50	0.5	(CropKing, 2025)
HydroCycle by FarmTek	Commercial production	115	540	0.8	(FarmTek, 2025)
VEVOR	Home production	5	32	0.6	(VEVOR, 2025)
Tower Garden	Home production	20	20	3.8	(TowerGarden, 2025)
Crop King	Home production	9.5	8	1.2	(CropKing, 2025)
Lettuce Grow	Home production	20	36	2	(Lettuce Grow, 2025)

The volume of NS can play a crucial role in nutrient management in recirculating hydroponic systems. This is because the initial volume of solution reflects nutrient availability, which plays a key role in nutrient availability throughout the growing cycle. Smaller volumes may lead to faster nutrient depletion and accumulation of more ions in the solution, while higher volumes can act as a buffer against nutrient fluctuations. Stable volume of NS is also necessary to prevent excessive ion accumulation (Velazquez-Gonzalez et al., 2022). A study by Miller et al. (2020) reported reduced N, P, K, and Fe levels and increased Na and Cu levels in the recycled. This resulted in lower shoot fresh weight and shoot water content, despite the maintenance of constant EC of $1.8 \text{ mS} \cdot \text{cm}^{-1}$, which can be related to the low volume of 20 L NS (1.3 L per plant).

Despite the importance of NS on nutrient availability, there is limited scientific research on optimizing NS volume in recirculating hydroponic systems. While EC-based nutrient management is widely used, EC alone is insufficient to maintain the nutrient balance over long cropping cycles without considering the volume of NS. Optimizing the NS volume per plant to ensure an adequate supply of nutrients (low NS volume) and reduce nutrient waste from over-supply (high NS volume) is an untested area of research in hydroponics production.

1.10 Future Research

Nutrient management is a crucial factor for enhancing the growth and quality of different vegetable species in hydroponic production. EC is one of the parameters that influence the growth and quality of crops produced in these systems. Numerous research studies have focused on lettuce, and growers generally follow the guidelines for lettuce for other leafy green species (Walters & Currey, 2015; Yang et al., 2024b). However, this can limit crop growth and quality of other leafy green species in a recirculating hydroponic system. In addition, if growers dump

out NS and refill weekly (Bugbee, 2004), nutrients are wasted and pollute the environment. At the same time, even maintaining EC at the target level, some nutrients such as N, P, K, and Fe become depleted, which may be due to insufficient NS volumes or how the NS is managed. Very limited research has focused on NS volume in recirculating hydroponic systems for leafy greens. Considering this importance, we hypothesized that there may be differences in plant growth, yield, tissue nutrients, and nutritional quality of different leafy green species based on NS volume per plant.

Therefore, the objectives of the research presented in this thesis are to explore different NS volumes and electrical conductivities:

1. To investigate the effect of different NS volumes on growth, yield, tissue nutrients, and nutritional quality of a variety of leafy green species during the summer and fall in greenhouse CEA; and
2. To investigate the effect of different EC levels on the growth, yield, and nutritional quality of different leafy green species in an indoor growth tent CEA.

These objectives are designed to provide growers with guidelines and insights for optimizing NS management for leafy greens in greenhouse and indoor production systems. This knowledge supports precise nutrient management for species-specific crop needs that ultimately can improve nutrient use efficiency, reduce nutrient waste, and promote sustainable hydroponic practices.

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Chapter 2 - Impact of Nutrient Solution Volume during Recirculating Hydroponic Production on Growth, Nutrient Content and Postharvest Characteristics of Four Leafy Greens

Species

Abstract

Efficient nutrient management is critical for optimizing hydroponic production. However, limited research exists on optimizing nutrient solution (NS) volume for various leafy greens vegetables in recirculating hydroponic cultivation. To address this gap, we evaluated partial nitrogen budgets, growth, nutrient responses and postharvest quality of four leafy greens species: butterhead lettuce (*Lactuca sativa* ‘Salanova Red Butter’), arugula (*Eruca sativa* ‘Standard’), kale (*Brassica oleracea* ‘Red Russian’), and the emerging crop Malabar spinach (*Basella alba* ‘Rubra’). These crops were grown in a nutrient film technique (NFT) hydroponic system under two NS volumes, low (2 L plant⁻¹) and high (4 L plant⁻¹), in a greenhouse during both summer and fall seasons. Electrical conductivity (EC) of 1.8 mS·cm⁻¹ and pH of 5.8 to 5.9 were maintained daily across treatments. Results showed that using a low NS volume resulted in higher N uptake efficiency and lower N retained in the NFT system in both seasons’ trials. NS volume had no impact on growth and yield parameters, but it influenced nutrient composition and quality attributes of leafy greens. Leaf nitrogen (N) and potassium (K) increased with high NS volume, while phosphorus (P) and K followed a similar trend in the fall. Low NS volume reduced nitrate levels in shoots of arugula, lettuce, and Malabar spinach during both seasons, suggesting that lower volume may help minimize excessive nitrate levels in plant shoots, while kale consistently surpassed recommended safe nitrate levels regardless of treatment.

Additionally, NS volume influenced key postharvest attributes of color, total phenolics, and antioxidant capacity. These findings highlight the value of crop-specific NS management to optimize plant health, improve nutrient use efficiency, minimize nitrate accumulation and nutrient waste, and support sustainable hydroponic production.

Keywords: antioxidants, arugula, kale, lettuce, Malabar spinach, nutrient budget, nutrient film technique, nutrient management, nitrate, phenolics, vitamin C

2.1 Introduction

Controlled environment agriculture (CEA) is a promising component of food production strategies that address the changing agricultural landscape and provide more localized food production in urban areas in an ecologically friendly manner (Dsouza et al., 2023; Engler & Krarti, 2021). CEA allows the control of environmental factors such as temperature, light, humidity, and CO₂; helps conserve water; reduces crop damage; and supports year-round production (Cowan et al., 2022; Gómez et al., 2019). Hydroponics, a key production system in CEA, is defined as the culture of growing plants in liquid nutrient solution instead of soil-based medium (Resh, 2022b; Shrestha & Dunn, 2017). Among the various production systems in CEA, hydroponics has gained wide attention for its efficient resource use and scalability.

Hydroponics offers benefits such as control over water and nutrient uptake, shorter production cycles, and no need for herbicides to control weeds (Bihari et al., 2023; Raviv et al., 2019; Sousa et al., 2024). Leafy greens are well-suited for hydroponic systems because of their short production cycle (Houston et al., 2023) and high market demand (Yang et al., 2024).

Beyond their agronomic fit, leafy greens also offer significant nutritional and health benefits. Lettuce, for instance, is low in calories, fat, and sodium, and an excellent source of vitamins, minerals, and bioactive compounds such as polyphenols, carotenoids, and chlorophyll with their related health benefits (Shi et al., 2022). Kale is a good source of fiber, minerals such as K and Calcium (Ca), prebiotic carbohydrates, unsaturated fatty acids, and different vitamin antioxidants, glucosinolates, and flavonoids (Satheesh & Workneh Fanta, 2020). Arugula has a pungent and bitter flavor due to the accumulation of glucosinolates and antioxidants and is also rich in P, S, Fe, vitamin A and C (Cavauiolo & Ferrante, 2014). Malabar spinach commonly known as vine spinach, Ceylon spinach, Indian spinach and climbing spinach. It is a heat-tolerant

leafy green widely consumed in Asia and was successfully cultivated in tropical regions such as Hawaii and Puerto Rico (Qiu & Liu, 2020). While it is not true spinach, it has several health benefits for instance, it is rich in vitamin A and C, carotenoids, phenols, alkaloids, tannins, bioflavonoids, organic acid, betacyanin, and minerals such as Ca, P, K, Mg (Qiu & Liu, 2020). With increasing consumer demand for diverse leafy green varieties, Malabar spinach offers expanded market potential in urban areas (Ernst, 2017). However, the species' production has not been studied extensively in hydroponics systems.

To achieve optimal growth and maximize the nutritional quality of leafy greens in hydroponic system, a balanced and consistent nutrient supply is essential (Meselmani, 2022). Inadequate or unbalanced mineral levels in the NS can cause deficiency or toxicity, which lead to physiological disorders that affect plant yield and quality (Chrysargyris & Tzortzakis, 2023; Singh et al., 2019).

In a recirculating hydroponic system, NS is recirculated and reused (Rouphael & Colla, 2005; Sanjuan-Delmás et al., 2020). Compared to linear or open production systems, these closed systems are commercially popular because of their benefits of higher water and nutrient use efficiency (Fayezizadeh et al., 2021; Rosa-Rodríguez et al., 2020). Recirculating hydroponic systems also support environmental sustainability by reducing the impact of the disposal of NS on the environment (Valenzano et al., 2008). However, maintaining nutrient stability over time can be challenging.

The volume of NS in these systems determines total initial nutrient availability for a crop, influences the rate of nutrient depletion, and affects the NS buffering capacity (Mullins, 2023). It also plays a critical role in managing ion accumulation, especially under closed-loop conditions where the same solution is reused throughout the crop cycle (Velazquez-Gonzalez et al., 2022).

In this context, water and nutrient replenishment strategies interact with the initial NS volume to influence overall plant performance.

Evidence suggests that suboptimal NS volume may lead to nutrient depletion or ionic imbalance even when electrical conductivity (EC) remains at a target level. For example, Miller et al. (2020) reported a reduction of N, P, K, and iron (Fe), while higher accumulation of sodium (Na) and copper (Cu) in recycled NS. This resulted in lower shoot fresh weight and shoot water content of lettuce, despite maintenance of constant electrical conductivity (EC) at $1.8 \text{ mS}\cdot\text{cm}^{-1}$. This may be related to a low volume of NS in the recirculating system, which was 20 L (1.3 L per plant).

Despite the importance of NS volume, no standards exist for optimizing volume of NS provided for each plant in a recirculating system. Hydroponic system manufacturers sell kits for the construction of recirculating NFT systems with NS tank size determined by a variety of factors. The volume of NS per plant in these systems ranges from 0.125 gallon (0.5 L) per plant (CropKing, 2025); 0.47 gallon (1.7 L) per plant for American Hydroponics (AmHydro, 2025), to 0.21 gallon (0.8 L) per plant for HydroCycle (FarmTek, 2025). The only recommendation found in the literature for commercial growers provided by Virginia Cooperative Extension was 0.25 to 1 gallon (1 to 3.8 L) per plant (Mullins, 2023). However, those available guidelines are based on experience rather than systematic scientific evaluation. As a result, commercial growers often rely on trial-and-error or generalized recommendations, which can compromise both production efficiency and environmental stewardship.

To address this knowledge gap, this research aims to systematically evaluate the effect of two different NS volumes on the growth, tissue nutrient content, and nutritional quality of four leafy green species cultivated in a recirculating NFT hydroponic system. In addition to the

frequently studied leafy greens of lettuce and basil, kale and Malabar spinach are also included. By identifying volume-specific responses, the overarching goal of this project is to improve nutrient use efficiency, minimize waste, and promote sustainable, high-quality food production in CEA systems.

2.2 Materials and Methods

2.2.1 Experimental Overview and Plant Materials

Two experiments were conducted in a greenhouse at Kansas State University's Olathe Horticulture Research and Extension Center (38.88° N, 94.99° W) during summer and fall seasons. Each experiment followed a split-plot experimental design with the volume of NS as the main plot and plant species as subplots. Two volumes of NS were low (75 L; 2 L per plant) and high (150 L; 4 L per plant), with EC and pH maintained daily, were used in each study. The four plant species were arugula (*Eruca sativa* 'Standard'), kale (*Brassica oleracea* 'Red Russian'), butterhead lettuce (*Lactuca sativa* 'Salanova Red Butter'), and Malabar spinach (*Basella alba* 'Rubra').

Seeds of the four plant species (Johnny's Selected Seeds, Albion, ME, USA) were sown in square agrifoam (Horticubes®; Smithers Oasis, Canton, OH, USA) using a small nutrient film technique (NFT) desktop system (53.34 cm L x 33.02 cm W x 15.24 cm H; CropKing, Lodi, OH, USA) under a photoperiod of 16 hours and a daily light integral (DLI) of 10 mol m⁻²·day⁻¹ and LED grow lights (Model 10101-10135A; Root Farm, Marysville, OH, USA). Healthy, uniform, and robust seedlings were transplanted after 14 to 15 days from sowing into the main NFT hydroponic (CropKing, Lodi, OH, USA) in a plastic-covered greenhouse chamber with an area of 80 m².

The main hydroponic NFT system consisted of six growing channels, each 1.2 m long, with six square holes (0.03 m × 0.03 m). These channels were spaced at 0.2 m intervals at the center, resulting in 36 plant spaces in each NFT system. A galvanized steel frame was used to support all the growing channels. A reservoir tank (Active aqua; Hydrofarm, Fairfield, Canada) of 40 gallons was equipped with a circulation pump (6.17 GPM, CropKing, Lodi, OH, USA) for transferring the NS to the growth channels and draining it back into the reservoir tank. Three systems were used for each of the NS volume treatments. Nine plants of each species were produced in each of the six NFT systems randomly across main plots (NFT systems).

Greenhouse temperature setpoints were 20°C for heating to 30°C for cooling and were regulated using an environmental controller (iGrow 800; Link 4 corporation, Anaheim, CA, USA). Actual environmental conditions during the summer (May to June 2024; Expt. 1) and fall (September to October 2024; Expt. 2) are reported in Table 2.1.

Table 2.1. Average temperature, relative humidity (RH), vapor pressure deficit (VPD), and dew point during the summer and fall experiment in greenhouse settings

Season	Av. Air Temperature (°C)	Av. RH (%)	Av. VPD (K Pa)	Av. Dew Point (°C)
Summer	24.5	66.3	1.2	16.9
Fall	20.6	70.8	1.1	13.7

2.2.2 Nutrient Solution Preparation

Fertilizer stock solution was prepared separately using a two-part water-soluble fertilizer (Hydro Grow Leafy Green Fertilizer, CropKing, Lodi, OH, USA) at 97.5 g·L⁻¹ and calcium nitrate (CropKing, Lodi, OH, USA) at 75 g·L⁻¹ as shown in Table 2.2.

Table 2.2. Composition of nutrients and their concentration in two-part fertilizer of leafy green vegetables

Nutrients	Composition	Concentration
	Hydro-Gro Leafy Greens (Part A)	
Macronutrients	Total N	4.3 %

	Available Phosphoric acid (P ₂ O ₅)	9.3 %
	Soluble Potash (K ₂ O)	35 %
	Magnesium (Mg)	3.9 %
Trace elements	Boron (B)	200 mg·L ⁻¹
	Cu	105 mg·L ⁻¹
	Fe	2100 mg·L ⁻¹
	Manganese (Mn)	190 mg·L ⁻¹
	Molybdenum (Mo)	42 mg·L ⁻¹
	Zinc (Zn)	210 mg·L ⁻¹
	Calcium Nitrate Ca (NO ₃) ₂ (Part B)	
Macronutrients	Total N	15.5 %
	Calcium (Ca)	19 %

To prepare different NS volumes, reservoirs were initially filled with 75% municipal water (EC: 0.37 mS·cm⁻¹, pH: 7.1), followed by adding an equal volume of stock solutions A and B to adjust the EC at 1.8 mS·cm⁻¹ within ±0.05. After this, additional water was added to bring the total volume to low or high volume based on treatment. EC was remeasured and confirmed at 1.8 mS·cm⁻¹ within ±0.05, and pH was maintained at 5.8-5.9. Both EC and pH were monitored daily using an EC and pH meter (HANNA[®] instruments, Smithfield, RI, USA) and recorded along with the temperature of NS, amount of stock solution or pH adjustment chemicals added. The NS volume was maintained at its original treatment volume each day throughout the experiment. pH was adjusted daily using 10% H₂SO₄ (diluted from 25% concentrated sulfuric acid) to lower the pH and 5% KOH (K hydroxide) to raise the pH. While maintaining NS volume treatments, EC was maintained daily by adding water to decrease EC and adding fertilizer stock solutions to increase EC.

2.2.3 Measurement of Plant Height, Number of Leaves, and SPAD

Plant height, leaf number, and SPAD were measured biweekly on all nine plants per species as an experimental unit. Plant height was determined by recording the distance from the base of the stem to the top of the foliage canopy using a ruler. The number of true leaves was counted for each plant. SPAD (an index of chlorophyll content per unit leaf area) for each plant was observed by measuring 3 to 4 fully expanded leaves at the central point between midrib and leaf margin and recorded using a chlorophyll meter (SPAD-502; Minolta Corporation, Ltd., Osaka, Japan).

2.2.4 Harvest and Measurement of Yield Parameters

All the yield parameters were measured on all nine plants per species as an experimental unit. In the summer trial, all leafy green vegetable species used in the experiment were harvested 24 days after transplanting, as they reached their harvest maturity index, while in the fall trial, harvesting occurred at 27 days. The shoot and root fresh weight were recorded (OHAUS, Parsippany, NJ, USA). Leaf area measurements were conducted on the day of harvest by assessing each leaf from every plant using (LI-3100C; LI-COR Environmental, Lincoln, NE, USA). Subsequently, shoot and root samples from each plant were promptly bagged in brown paper bags and oven-dried at 75 °C until constant weight. Then shoot and root dry weight were recorded. The shoot and root water content were calculated using Equation (2.1), and the percentage shoot and root dry matter content using Equation (2.2), following the method described by Vaieretti et al. (2007).

Equation 2.1. The percentage of shoot and root water content

$$\frac{(FW-DW)}{FW} \times 100$$

Equation 2.2. The percentage of shoot and root dry matter content

$$\frac{DW}{FW} \times 100$$

where *FW*: fresh weight of either shoot or root, *DW*: dry weight of either shoot or root.

2.2.5 Measurement of Nutrients in Nutrient Solution

A 45 ml water sample was collected at 0, 14, and 24 days during the summer trial and at 0, 14, and 27 days during the fall trial, and was sent to the Soil Testing Laboratory at Kansas State University, Manhattan, KS, USA for total N, P, K, and nitrate analysis. For the total N and P analysis, 1 to 10 ml of each sample was digested with K persulfate reagent in an autoclave (Hosomi & Sudo, 1986; Nelson, 1987). The digested samples were then analyzed for nitrate N concentration using the cadmium reduction method based on Alpkem Rapid Flow Analysis (RFA) Methodology No. A303-S170 (Alpkem Corporation, 1986), while orthophosphate was measured according to Alpkem Rapid Flow Analysis (RFA) Methodology A303-S200-13 (Alpkem Corporation, 1986). In addition, analysis of K in water was performed using an Inductively Coupled Plasma (ICP) Spectrometer (Model 5800 ICP OES, Agilent Technologies).

2.2.6 Measurement of Nutrients in Plant Tissue

All dried shoot samples, including shoot and leaves were ground using a mill (Cyclotec™ 1093; FOSS Analytical, Hillerød, Denmark) and sent to the Soil Testing Laboratory for analysis of total N, P, K, and nitrate concentration in the plant tissue.

Total N, P, and K in plant tissue were analyzed using the sulfuric peroxide digestion method (Lindner & Harley, 1942; Thomas et al., 1967). The resulting digest, which contains ammonia, was analyzed by an indophenol blue colorimetric procedure using the Rapid Flow Analyzer (Model RFA-300) from Alpkem Corporation (Clackamas, OR, USA), following RFA Methodology No. A303-S072. P and K concentrations were determined using an Inductively

Coupled Plasma (ICP) Spectrometer meter (Model 5800 ICP OES; Agilent Technologies, Santa Clara, CA, USA).

2.2.7. Calculation of Partial Nitrogen Budget

Partial N budgets were calculated by assessing total N inputs, estimated N uptake efficiency, N recovery by plants, and N remaining in the system for each NS volume treatment, as follows.

Equation 2.3. Total amount of N input

Total N input in mg = Nitrogen concentration in stock A and B × total volume of stock A and B added

Equation 2.4. Estimated percentage of N uptake

$$\text{Estimated \% N uptake} = \frac{N \text{ uptake (mg)}}{N \text{ input (mg)}} \times 100$$

Equation 2.5. Amount of N recovered in plant tissues

N recovery per tank in mg = Total N shoot and root tissue concentrations per tank in mg

Equation 2.6. Amount of N left in a system

N retention per tank in mg = Total N input (mg) - Total N recovery (mg)

2.2.8 Color and Texture

On harvest day, the fresh shoot samples were transported to the Postharvest Physiology lab at K-State Olathe in an air-conditioned vehicle. Surface color was determined based on three plants per species for each treatment replication and measured on four outer leaves of each plant. Color was measured using a chromameter (A5 CR-400; Minolta Co.Ltd., Osaka, Japan) and was expressed in the International Commission on Illumination (CIE) L*a*b* color space, where a* denotes the red/green value and L* indicates lightness. And hue angle (h°) was calculated following the procedure of Setser (1984). Hue angle was expressed on a 360° color wheel where 0° and 360° represent red, 270° represents blue, 180° represents green, and 90° represents yellow.

Texture was measured on three plants per species for each treatment replication. Five outer leaves without midrib from each replication for lettuce, three outer leaves with midrib from each replication for arugula, and three outer leaves without midrib from each replication for kale were determined for texture. The texture was not performed for Malabar spinach because of an inadequate number of leaves. Textures were measured using a texture analyzer (TA-58, TA.XT.plus, Texture Technologies Corp., Scarsdale, NY, USA) with a Kramer Shear Cell (TA-91; Texture Technologies Corp., Scarsdale, NY, USA) equipped with five blades. Leaf texture was calculated as the maximum force in Newton per gram (Batziakas et al., 2020).

2.2.9 Nutritional Quality

Nutritional quality, including phytochemicals and vitamin C was determined by using three fresh plant shoot samples per species (arugula, kale, lettuce and Malabar spinach) for each treatment replication. Plant samples were cut into pieces with a knife and homogenized. For each treatment replication, two samples of 40 grams of fresh leaves per species were collected for anthocyanin, total phenolics and antioxidant capacity. The collected samples were frozen and stored at -20°C for further analysis. The frozen samples were freeze-dried using a freeze dryer (Harvest Right, North Salt Lake, UT, USA). All samples were finely pulverized into powder using a pestle and mortar in liquid N and transferred into centrifuge tubes for the following analysis.

2.2.9.1 Phytochemicals

Anthocyanin content was measured only in lettuce, as red butter lettuce has a vibrant red color rich in anthocyanin concentration (Anum et al., 2024). It was measured following the procedure provided by Adamse et al. (1989), Gazula et al. (2007), and Kleinhenz et al. (2003). Stored lettuce samples were immediately frozen in liquid nitrogen after harvest. Approximately

0.2 g of powder was transferred to a 15 ml centrifuge tube and extracted using 10 ml of methanol solution. The mixture was vortexed for 10 seconds and kept overnight at -20°C. On the following day, extracted samples were vortexed for 10 seconds and transferred into 1.75 ml tube and subsequently centrifuged (JA-17; Beckman Coulter, Palo Alto, CA, USA) for 10 minutes at 4°C at 8,000 rpm. The extract was diluted with 1% methanol. A final volume of 800 µl per diluted sample was prepared and vortexed. A volume of 250 µl from each sample and standard solution was plated in triplicate on a 96-well microplate. The absorbance was measured at 550 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Anthocyanin concentrations were calculated by using a standard curve generated with cyanidin-3-glucoside (C₃G) in 1% acidified methanol and were expressed as mg C₃G·100 g⁻¹ DW.

Total phenolics was measured for all four plant species by following the procedure described by Singleton and Rossi (1965). Stored plant samples were immediately frozen in liquid nitrogen after harvest. Approximately 0.2 g of powder was transferred to a 15 ml centrifuge tube and extracted using 10 ml of acetone and deionized water (v:v=1:1). The mixture was vortexed for 10 seconds and kept overnight at -20°C. On the following day, extracted samples were vortexed for 10 seconds and 100 µl was transferred into 1.75 ml tube. Subsample was diluted based on species using acetone and deionized water (v:v=1:1) for a final volume of 100 µl. Then, 500 µl of dilute Folin-Ciocalteu reagent was added to each sub-sample and standard solutions, followed by 400 µl of sodium carbonate after 0.5-8 min. Subsamples were vortexed and incubated in a dryer at 30 °C for one hour. Then, it was centrifuged at 3,000 rpm for 5 min at 4 °C. A 250 µl of each sample and standard solutions was plated in triplicate on a 96-well microplate. The absorbance was measured at 760 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Total phenolics were calculated by using a

standard curve generated with gallic acid equivalents (GAE) and were expressed as mg GAE·100 g⁻¹ DW.

Antioxidant capacity was analyzed for all four species using FRAP (Ferric Reducing Ability of Plasma) methods following the procedures described by Benzie & Strain (1996) and Benzie & Szeto (1999). The same extraction procedure used for total phenolics was followed. On the analysis day, extracted samples were vortexed for 10 seconds and transferred into a 1.75 ml tube. It was centrifuged at 11,300 rpm for 20 min at 4 °C. Centrifuged extract was diluted based on the species in acetone and deionized water (v:v=1:1). For the assay, 200 µl of sample extract was diluted to 720 µl of fresh work reagent (10 mM TPTZ: Tripyridyltriazine, 20 mM FeCl₃: Ferric chloride, and 300 mM sodium acetate buffer (pH 3.6) in a 1:1:10 ratio). A 230 µl of each sample and standard solutions were plated in triplicate on a 96-well microplate. The absorbance was measured at 593 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Antioxidant capacity were calculated by using a standard curve and was expressed as µmol TE (Trolox equivalents)·100 g⁻¹ DW.

Vitamin C, which is also known as ascorbic acid (AA), was measured all four plant species by following the procedure described by Klimczak & Gliszczynska-Świgło (2015) and Desai & Desai (2019). A 2 g of homogenized samples from three plant samples per species per treatment were collected and mixed with 20 ml of acid solution (2 N glacial acetic acid and 6% metaphosphoric acid) using a benchtop homogenizer (POLYTRON PT 1600 E; Kinematica Inc., Bohemia, NY, USA). The samples were stored at -20°C for further analysis. On the analysis day, samples were thawed and centrifuged at 12,000 rpm for 20 min at 4°C and again centrifuged for clarification. A 1 ml of clarified extract was used for the analysis of ascorbic acid (AA). To oxidize Ascorbic Acid (AA) to dehydroascorbic acid (DHAA), a drop of 0.2% of 2,6-

dichloroindophenol (2,6-DCIP) solution was added, followed by incubation for 1 hour at room temperature. A 2% thiourea solution was added to precipitate the excess 2,6-DCIP, and then 2% 2,4-dinitrophenylhydrazine (2,4-DNPH) solution was added. Samples and standard solutions were incubated at 60°C for 3 hours in an oven (Heratherm™ Convection, ThermoFisher Scientific Inc., Asheville, NC, US) to allow the formation of ozone. After incubation, samples and standard solutions were cooled in an ice bath and reacted with 90% sulfuric acid. Absorbance was measured at 540 nm using a plate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA) by following the procedure described by Terada et al. (1978). Vitamin C was quantified using a standard calibration curve and expressed as $\text{mg} \cdot 100 \text{ g}^{-1} \text{ FW}$.

2.2.10 Statistical Analysis

Statistical analysis was performed using software (SAS version 9.4, Cary, NC, USA). Statistical analyses were conducted for summer and fall trials separately. In each trial, a split-plot model was fitted through PROC GLIMMIX for each of the responses. The two levels of NS volume are the main plots. The four levels of species are subplots. Both the main effects and interaction effects are evaluated using Type III F-tests. Further, Comparisons are carried out using LSMEANS. A two-sided t-test at the level of 0.05 with Tukey's adjustment is applied to the pairwise comparisons. Additionally, for the growth data, the week variable reflects the time effect. Both the main effects and interaction effects are evaluated using Type III F-tests and further sliced by the time variable.

2.3. Results

2.3.1 Partial N Budgets in Different Nutrient Solution Volumes

Nitrogen uptake efficiency was significantly influenced by NS volume (Figure 2.1). In the summer trial, no significant difference was observed in week 2 (W2). However, by week 4

(W4), N uptake efficiency in low volume was 64% higher compared to high volume treatment. In contrast, during the fall trial, low volume had significantly higher N uptake efficiency by 458% compared to high volume treatment. However, by W4, there was no difference in the N uptake efficiency between treatments.

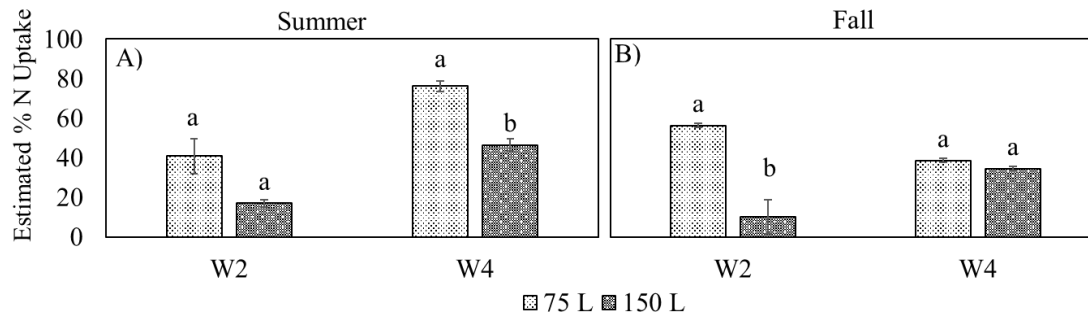


Figure 2.1. Estimated % N uptake by hydroponically grown leafy greens in the recirculating hydroponic system with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) at week 2 (W2) and week 4 (W4) during the summer (A) and fall (B) trials in the greenhouse

The total N input in high volume treatment increased by 87% during the summer trial and by 61% during the fall trial compared to low volume (Figure 2.2). However, no differences in N recovery rate in plant tissues were observed between the treatments during the summer and fall trials. In contrast, the N retention rate in high volume treatment increased by 113% in the summer trial and 83% in the fall trial compared to the low volume.

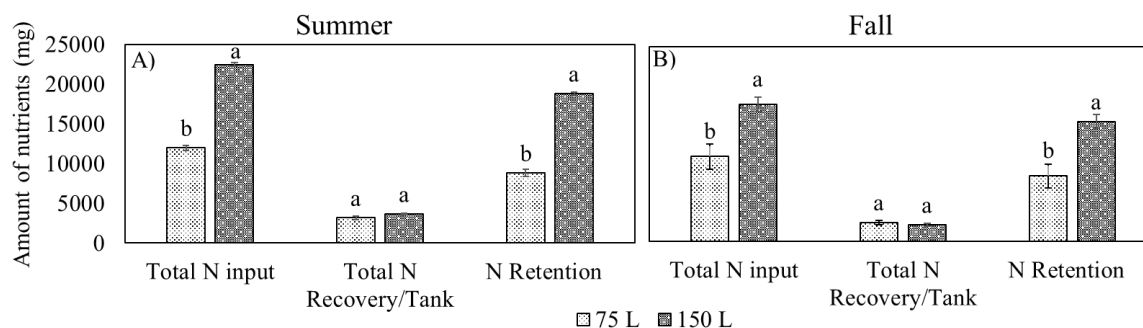


Figure 2.2. Total N input, total N recovery per tank, and nutrient retention in the recirculating hydroponic system with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer (A) and fall (B) trials in the greenhouse

2.3.2 Plant Height, Leaf Number, and SPAD in Different Nutrient Solution Volumes

Pooled data across species was analyzed to assess the main effect of NS volume on plant height, leaf number, and SPAD during both summer and fall trials (Figure 2.3). The results reflect the main effect of NS volume on these parameters at W2 and W4 during both the summer and fall trials. No significant differences in plant height, leaf number, or SPAD were observed within each week in either trial ($P>0.05$). Figure 2.4 presents the interaction effect of NS volume and species on plant height, leaf number, and SPAD for arugula, kale, lettuce, and Malabar spinach at W2 and W4. There were no significant differences between the low and high volume treatments, nor among the species, for any parameter measured during either the summer or fall trial ($P>0.05$).

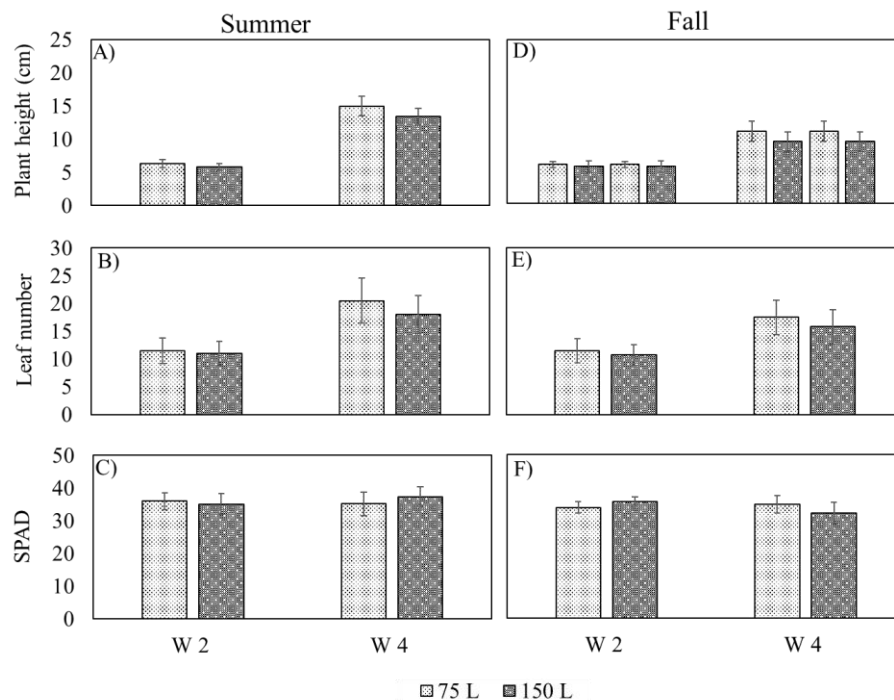


Figure 2.3. Average plant height (A), leaf number (B) and SPAD (C) during the summer trial and plant height (D), leaf number (E) and SPAD (F) during the fall trial with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) at week 2 (W2) and week 4 (W4) in the greenhouse

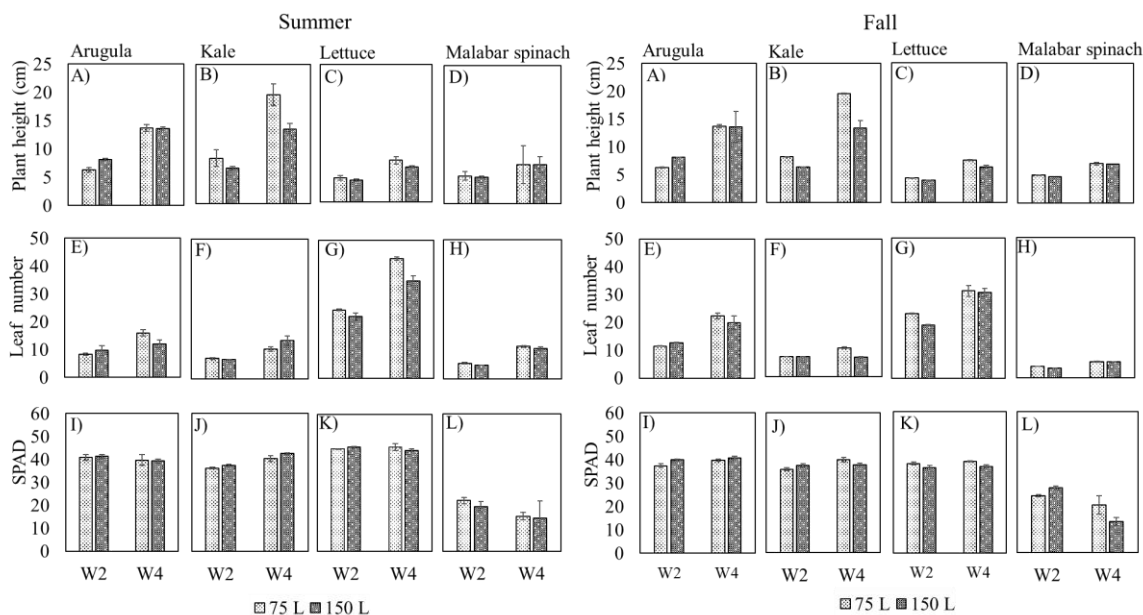


Figure 2.4. Average plant height, leaf number and SPAD with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) at week 2 (W2) and week 4 (W4) across arugula ‘Standard’, kale ‘Red Russian’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ during the summer and fall trials in the greenhouse

2.3.3 Plant Weight and Leaf Area in Different Nutrient Solution Volumes

Pooled data across species was analyzed to evaluate the main effect of NS volume on shoot and root fresh and dry weights and leaf area during both summer and fall trials (Table 2.3). During the summer trial, there were no significant differences between the low and high volume treatments for any parameter measured ($P>0.05$). During the fall trial, a significant difference was observed only for root dry weight ($P<0.01$), where the low volume treatment increased root dry weight by 22% compared to the high volume treatment. All other parameters in the fall trial were unaffected by NS volume.

Table 2.4 presents the effects of NS volume, species, and their interactions on shoot and root fresh weight, shoot and root dry weights, and leaf area during both trials. A significant interaction between species and NS volume was found for shoot fresh weight during the summer trial ($P<0.05$). Kale in the high volume treatment showed the highest shoot fresh weight, which

was 139% greater than lettuce in the same treatment, and 189% and 453% greater than Malabar spinach under low and high volumes, respectively. However, the effect of NS volume on shoot fresh weight was not statistically significant within any individual species. Though not statistically different, shoot fresh weight numerically increased for arugula and kale, and decreased for lettuce and Malabar spinach in the high volume treatment (Figure 2.5). During the fall trial, no significant interaction effects were observed for shoot fresh weight ($P>0.05$). However, shoot fresh weight numerically decreased in the high volume treatment across all four species. No significant interaction effects were found for root fresh weight, shoot and root dry weights, or leaf area in either trial ($P>0.05$).

Table 2.3. Average shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) and leaf area with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer and fall trials in the greenhouse

Season	Volume (L)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	Leaf area (cm ²)
Summer	75	124.3	32.6	6.9	1.3	1185
	150	123.0	30.3	6.8	1.2	1205
P-value		NS ⁱⁱ	NS	NS	NS	NS
Fall	75	84.8	27.5	5.6	1.1a ⁱ	895
	150	70.7	27.4	4.7	0.9b	800
P-value		NS	NS	NS	**	NS

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)

ⁱⁱNS, *, ** mean non-significant or significant at $p \leq 0.05, 0.01$, respectively

Table 2.4. Average shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) and leaf area with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula 'Standard', kale 'Red Russian', butterhead lettuce 'Salanova Red Butter' and Malabar spinach 'Rubra' in greenhouse settings during the summer and fall trials in the greenhouse

Season	Species	Volume (L)	SFW (g)	RFW (g)	SD (g)	RDW (g)	Leaf Area (cm ²)
Summer	Arugula (n=9) ⁱ	75	134.4abc ⁱⁱ	43.1	9.7	1.8	1245
		150	169.6ab	50.0	10.6	1.8	1525
	Kale (n=9)	75	151.4abc	41.2	12.3	1.8	1525
		150	201.3a	35.4	11.9	1.4	1529
	Lettuce (n=9)	75	141.9abc	23.6	3.2	0.7	1450
		150	84.3bcd	17.5	3.0	0.7	1505
	Malabar spinach (n=9)	75	69.7cd	22.7	2.2	1.0	520

		150	36.4d	18.9	1.6	0.9	261
Volume			NS ⁱⁱⁱ	NS	NS	**	NS
Species			***	***	***	***	***
Volume×Species			*	NS	NS	NS	NS
Fall	Arugula (n=9)	75	96.5	41.6	7.4	1.6	864
		150	79.7	33.0	5.8	1.2	797
	Kale (n=9)	75	164.3	40.9	11.6	1.8	1436
		150	137.9	48.0	10.4	1.3	1602
	Lettuce (n=9)	75	70.5	21.4	3.0d	0.8	1211
		150	58.0	21.5	2.2d	0.7	722
	Malabar spinach (n=9)	75	7.9	5.7	0.26	0.4	68
		150	7.5	7.6	0.24	0.4	78.
Volume			NS	NS	NS	NS	NS
Species			***	***	***	***	***
Volume×Species			NS	NS	NS	NS	NS

ⁱ n=9 total number of samples used per treatment of the reported average

ⁱⁱ Means within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$)

ⁱⁱⁱ NS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

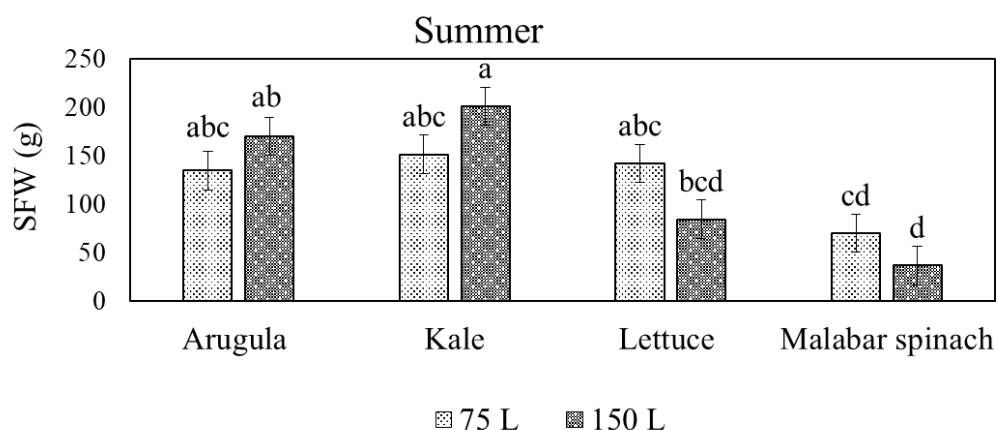


Figure 2.5. Average shoot fresh weight (SFW) with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula ‘Standard’, kale ‘Red Russian’, butterhead lettuce ‘Salanova Red Butter’, and Malabar spinach ‘Rubra’ during the summer trial in the greenhouse

2.3.4 Water Content and Percent Dry Matter Content in Different Nutrient Solution

Volumes

Pooled data across all species was analyzed to evaluate the main effect of NS volume on shoot and root water content and percent shoot and root dry matter content (Table 2.5). During the

summer and fall trials, no significant differences were observed between low and high volume treatments for shoot and root water content and percent shoot and root dry matter content ($P>0.05$).

Table 2.6 displays the effect of volume, species, and their interactions on shoot and root water content and percent shoot and root dry matter content during the summer and fall trials. No significant interaction effects were observed for shoot and root water content and percent shoot and root dry matter content ($P>0.05$) in either season.

Table 2.5. Average shoot water content (SWC), root water content (RWC) and percentage shoot dry matter content (SDM), and root dry matter content (RDM) with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer and fall trials in the greenhouse

Season	Volume (L)	SWC (%)	RWC (%)	SDM (%)	RDM (%)
Summer	75	94.2	95.8	6.1	4.2
	150	94.8	95.8	5.5	4.2
P-value		NS	NS	NS	NS
Fall	75	94.3	95.3	6.1	4.7
	150	94.6	96.2	5.6	3.8
P-value		NS	NS	NS	NS

NS means non-significant at $p \leq 0.05$

Table 2.6. Average shoot water content (SWC), root water content (RWC) and percentage shoot dry matter content (SDM), and root dry matter content (RDM) with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula ‘Standard’, kale ‘Red Russian’, butterhead lettuce ‘Salanova Red Butter’, and Malabar spinach ‘Rubra’ in greenhouse settings during the summer and fall trials in the greenhouse

Season	Species	Volume (L)	SWC (%)	RWC (%)	SDM (%)	RDM (%)
Summer	Arugula (n=9) ⁱ	75	92.5	95.9	7.5	4.1
		150	93.1	96.3	6.9	3.7
	Kale (n=9)	75	90.7	95.4	9.4	4.6
		150	94.0	95.8	6.0	4.2
	Lettuce (n=9)	75	97.0	96.4	3.0	3.6
		150	95.8	95.8	4.0	4.2
	Malabar spinach (n=9)	75	96.7	95.6	4.7	4.4
		150	94.9	95.2	5.1	4.8
	Volume		NS ⁱⁱ	NS	NS	NS
	Species		**	NS	**	NS
	Volume×Species		NS	NS	NS	NS
Fall	Arugula (n=9)	75	92.4	96.0	7.6	4.0
		150	92.2	96.4	7.8	3.6
	Kale (n=9)	75	92.4	95.4	7.6	4.6
		150	92.5	96.6	7.5	3.4
	Lettuce (n=9)	75	95.8	96.2	4.2	3.8
		150	96.7	96.7	3.9	3.3

	Malabar spinach (n=9)	75	96.9	93.5	4.9	6.5
		150	96.8	93.1	3.2	4.9
Volume			NS	NS	NS	NS
Species			****	**	***	**
Volume×Species			NS	NS	NS	NS

ⁱReported value are the average of n=9 repetitions of each treatment

ⁱⁱNS, *, **, **** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001 respectively

2.3.5 Shoot Mineral Nutrient Concentration in Different Volumes of Nutrient

Solution

Pooled data across species were analyzed to evaluate the main effect of NS volume on shoot N, P, K and nitrate concentrations during both summer and fall trials (Table 2.7). During the summer trial, NS volume had a significant main effect on shoot N ($P < 0.01$) and K (< 0.05), where the high volume treatment increased shoot N and K by 12% and 33%, respectively, compared to the low volume treatment. No significant difference in nitrate concentration was observed between treatments, although a numerical increase of 22% was observed in the high volume treatment. In contrast, during the fall trial, NS volume had a significant main effect on shoot P and K ($P < 0.05$), where the high volume treatment increased shoot P and K by 14% and 6%, respectively, compared to the low volume treatment. Nitrate concentration also showed no statistical difference but increased numerically by 20% in the high volume treatment.

Table 2.8 displays the shoot N, P, K, and nitrate concentrations among species during both trials. During the summer trial, nitrate concentration varied significantly among species ($P < 0.01$), with arugula and kale having the highest levels, followed by lettuce and Malabar spinach. No significant differences were observed among species for shoot N, P, and K. In contrast, during the fall trial, significant variation was observed among species for all four parameters ($P < 0.001$). Lettuce had the highest N concentration, statistically similar to arugula and kale, but 26% higher than Malabar spinach. For P, lettuce again had the highest

concentration, similar to kale and Malabar spinach, but 33% higher than arugula. Arugula had the lowest K concentration, approximately 29.3%, 28.5% and 14.5% lower than Malabar spinach, lettuce and kale. Nitrate concentration was highest in arugula, exceeding lettuce and Malabar spinach by 180% and 210%, respectively. Table 2.9 presents the effect of volume, species, and their interaction on shoot N, P, K, and nitrate concentrations. No significant interaction effects were observed during either trial ($P>0.05$).

Table 2.7. Average tissue nitrogen (N), phosphorus (P), potassium (K), and nitrate content with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer and fall trials in the greenhouse

Season	Volume (L)	N (% of DW) ⁱ	P% (% of DW)	K (% of DW)	Nitrate (mg·kg ⁻¹ FW)
Summer	75	5.0b ⁱⁱ	1.0	6.0b	3401
	150	5.6a	0.8	8.0a	4147
P-value		**	NS	*	NS
Fall	75	4.7	0.7b	7.9b	3290
	150	5.0	0.8a	8.4a	3956
P-value		NS ⁱⁱⁱ	*	*	NS

ⁱN, P and K content was expressed in % based on dry weight (DW) basis

ⁱⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively.

Table 2.8. Average tissue nitrogen (N), phosphorus (P), potassium (K), and nitrate content among arugula 'Standard', kale 'Red Russian', butterhead lettuce 'Salanova Red Butter', and Malabar spinach 'Rubra' during the summer and fall in the greenhouse

Season	Species	N (% of DW) ⁱ	P% (% of DW)	K (% of DW)	Nitrate (mg·kg ⁻¹ FW)
Summer	Arugula	5.3	0.9	5.7	4793a
	Kale	5.6	0.9	7.2	5624a
	Lettuce	5.4	0.7	7.9	2667b
	Malabar spinach	4.8	1.1	7.1	2013b
P-value		NS	NS	NS ⁱⁱⁱ	**
Fall	Arugula	5.0a ⁱⁱ	0.6b	6.5c	5529a
	Kale	4.8a	0.7ab	7.6b	5213a
	Lettuce	5.3a	0.8a	9.1a	1973b
	Malabar spinach	4.2b	0.7ab	9.2a	1778b
P-value		*** ⁱⁱⁱ	***	***	***

ⁱN, P and K content was expressed in % based on dry weight (DW) basis

ⁱⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001 respectively.

Table 2.9. Average tissue nitrogen (N), phosphorus (P), potassium (K), and nitrate content with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula ‘Standard’, kale ‘Red Russian’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ in greenhouse settings during the summer and fall trials in the greenhouse

Season	Species	Volume (L)	N (% of DW) ⁱ	P% (% of DW)	K (% of DW)	Nitrate (mg·kg ⁻¹ FW)	
Summer	Arugula (n=4) ⁱⁱ	75	4.7	1.3	4.4	4534	
		150	6.0	0.7	7.1	5053	
	Kale (n=4)	75	5.3	0.8	6.9	6083	
		150	5.9	0.9	7.5	5166	
	Lettuce (n=4)	75	5.4	0.7	7.7	1574	
		150	5.5	0.8	8.2	3758	
	Malabar spinach (n=4)	75	4.5	1.3	5.1	1412	
		150	5.1	0.9	9.0	2613	
	Volume			**iv	NS	*	NS
	Species			NS	NS	NS	**
Volume×Species			NS	NS	NS	NS	
Fall	Arugula (n=4)	75	5.0	0.7	6.5a	4590	
		150	5.0	0.6	6.6a	6467	
	Kale (n=4)	75	4.8	0.7	7.5a	4991	
		150	4.9	0.8	7.8a	5433	
	Lettuce (n=4)	75	5.1	0.8	9.0a	2046	
		150	5.5	0.9	9.1a	1899	
	Malabar spinach (n=4)	75	3.9	0.7	8.4b ⁱⁱⁱ	1532	
		150	4.4	0.8	10.0a	2022	
	Volume			NS	*	*	NS
	Species			***	***	***	***
Volume×Species			NS	NS	NS	NS	
			Sufficiency range ^v			Safe range ^{vi}	
(Bryson & Mills, 2014; N. Mattson & Merrill, 2015) (EU Commission Regulation, 2011)							
	Arugula		2.86-3.97	0.61-0.72	4.80-5.16	6000-7000	
	Kale		2.96-5.14	0.31-0.68	2.55-4.04	500-1000	
	Lettuce		4.50-6.50	0.30-0.80	6.0-10.0	1000-2500	

ⁱ N, P, and K content was expressed in % based on dry weight (DW) basis

ⁱⁱ n=4 total number of samples used per treatment of the reported average

ⁱⁱⁱ Means within a column followed by the same letter are not significantly different based on Tukey’s honestly significant difference

test ($\alpha=0.05$)

^{iv} NS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

^v Leaf tissue sufficiency range for the NPK for each species (Bryson & Mills, 2014); comparison to whole shoot tissue (leaves and stems)

^{vi} Safe range provided by European Commission (EU) regulations No 1258/2011

2.3.6 Color in Different Nutrient Solution Volumes

Pooled data across species was analyzed to evaluate the main effect of NS volume on color indices (L^* , a^* and h°) both summer and fall trials (Table 2.10). Significant differences were observed between the low and high volume treatments for L^* ($P<0.05$), and for a^* and hue angle ($P<0.001$). During the fall trial, the high volume treatment resulted in a 9% increase in L^* and a 50% increase in a^* , while a 21% decrease in h° compared the low volume treatment.

Table 2.11 displays the color indices across the species. Significant differences were observed among species for L^* and a^* ($P<0.001$), and for h° ($P<0.01$). Arugula had the highest L^* values, which were 43.6% and 42.8% higher than those of lettuce and kale, respectively, but had similar L^* value to Malabar spinach. Lettuce had the highest a^* values, which were 51% and 49% higher than those of arugula and Malabar spinach, respectively. Similarly, h° was highest in arugula, differing from kale and lettuce by 7.2% and 7.1%, respectively, but had a similar h° value to Malabar spinach.

Table 2.12 displays the effect of volume, species, and their interactions on color indices. Significant interactions were observed for all three parameters ($P<0.001$). In the high volume treatment, L^* increased by 45% in arugula and 50% in Malabar spinach, whereas it decreased by 31% in kale and 27% in lettuce compared to the low volume treatment. The a^* value increased by 93% in kale and 95% in lettuce in the high volume treatment compared to low volume. Conversely, h° was higher in the low volume treatment, with increases of 16% in arugula, 40% in kale, 41% in lettuce, and 14% in Malabar spinach compared to the high volume treatment.

Table 2.10. Average color indices (L^* , a^* , and h°) with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer and fall trials in the greenhouse

Season	Volume (L)	L^*	a^*	h°
Fall	75	48.0b ⁱ	-17.5b	127.1a
	150	52.1a	-8.7a	100.4b

P-value	*ii	***	***
ⁱ Means within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)			
ⁱⁱ NS, *, **, *** mean non-significant or significant at $p \leq 0.05, 0.01$ or 0.001 , respectively			

Table 2.11. Average color indices (L*, a*, and h°) among arugula 'Standard', kale 'Red Russian', butterhead lettuce 'Salanova Red Butter', and Malabar spinach 'Rubra' during the summer and fall in the greenhouse

Species	L*	a*	h°
Arugula	59.3a ⁱ	-16.9b	117.7a
Kale	41.5b	-8.7a	109.8b
Lettuce	41.3b	-8.6a	109.9b
Malabar spinach	58.1a	-17.5b	117.5a
P-value	*** ⁱⁱ	***	**

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)

ⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05, 0.01$ or 0.001 , respectively

Table 2.12. Average color indices (L*, a*, and h°) with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula 'Standard', kale 'Red Russian', butterhead lettuce 'Salanova Red Butter' and Malabar spinach 'Rubra' in greenhouse settings during the summer and fall trials in the greenhouse

Season	Species	Volume (L)	L*	a*	h°
Fall	Arugula	75	48.5b ⁱ	-17.8b	126.2a
		150	70.1a	-16.0b	109.3b
	Kale	75	49.3b	-16.1b	128.1a
		150	33.8c	-1.2a	91.4c
	Lettuce	75	46.b	-16.7b	128.6a
		150	34.9c	-0.9a	91.1c
	Malabar spinach	75	46.4b	-18.4b	125.23a
		150	69.8a	-16.7b	109.8b
	P-value			*** ⁱⁱ	***

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)

ⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05, 0.01$ or 0.001 , respectively

2.3.7 Texture, Vitamin C, Anthocyanin, Total Phenolics, and Antioxidant Capacity in Different Nutrient Solution Volumes

Pooled data across species was analyzed to evaluate the main effect of NS volume on texture, vitamin C, anthocyanin, total phenolics, and antioxidant capacity during both summer

and fall trials (Table 2.13). During the summer trial, NS volume had a significant difference on total phenolics and antioxidant capacity ($P < 0.05$), where the low volume treatment increased total phenolics and antioxidant capacity by 59% and 57% higher, respectively, compared to the high volume treatment. In contrast, vitamin C and texture remained unaffected by NS volume. During the fall trial, all parameters were not influenced by NS volume.

Table 2.14 summarizes the species-level effects on texture, vitamin C, total phenolics, and antioxidant capacity during both trials. During the summer trial, significant differences were observed among species for texture ($P < 0.001$) and vitamin C ($P < 0.01$). Kale exhibited the firmer texture value (greater firmness) than arugula and lettuce by 97% and 306%, respectively. Arugula had the highest vitamin C concentration, comparable to kale and Malabar spinach, and was 207% higher than that of lettuce. In the fall trial, species had a significant effect on vitamin C ($P < 0.001$), antioxidant capacity ($P < 0.001$), and total phenolics ($P < 0.01$). Arugula again had the highest vitamin C concentration, which was also comparable to kale and Malabar spinach, and was 215% higher than that of lettuce. Lettuce showed the highest antioxidant capacity, exceeding that of arugula, kale, and Malabar spinach by 240%, 155%, and 137%, respectively. Similarly, lettuce had the highest total phenolics content, which was 73%, 90%, and 58% higher than those of arugula, kale, and Malabar spinach, respectively. Table 2.15 displays the effect of volume, species, and their interactions on texture, vitamin C, total phenolics, and antioxidant capacity. No significant interaction effects were observed during the summer or fall trials. However, during the summer trial, lettuce showed a 148% higher antioxidant capacity in the high volume treatment compared to the low volume.

Table 2.13. Average texture, anthocyanin, vitamin C, antioxidant capacity, and total phenolics content with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) during the summer and fall experiment in the greenhouse

Season	Volume (L)	Texture (N·g ⁻¹)	Anthocyanin ⁱ (mg·100 g ⁻¹ DW)	Vitamin C (mg·100 g ⁻¹ FW)	Antioxidant Capacity (μmol TE·100 g ⁻¹ DW)	Total Phenolics (mg GAE·100 g ⁻¹ DW)
Summer	75	43.6	125.3	65.4	4.6a ⁱⁱ	1.1a
	150	39.5	74.3	57.7	2.9b	0.7b
P-value		NS ⁱⁱⁱ	NS	NS	*	*
Fall	75	13.1	411.9	75.8	4.9	1.2
	150	12.7	363.8	73.9	5.8	1.4
P-value		NS	NS	NS	NS	NS

ⁱAnthocyanin concentrations were determined for lettuce only

ⁱⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference

test ($\alpha = 0.05$)

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 2.14. Average texture, vitamin C, antioxidant capacity, and total phenolics content among arugula 'Standard', kale 'Red Russian', butterhead lettuce 'Salanova Red Butter', and Malabar spinach 'Rubra' during the summer and fall in the greenhouse

Season	Species	Texture (N·g ⁻¹)	Vitamin C (mg·100 g ⁻¹ FW)	Antioxidant Capacity (μmol TE·100 g ⁻¹ DW)	Total Phenolics (mg GAE·100 g ⁻¹ DW)
Summer	Arugula	36.1b ⁱ	83.7a	3.2	1.0
	Kale	71.1a	63.7a	4.0	0.8
	Lettuce	17.5c	27.3b	4.7	0.9
	Malabar spinach	- ⁱⁱ	71.5a	3.2	0.9
	P-value	*** ⁱⁱⁱ	**	NS	NS
Fall	Arugula	13.2	100.9a	3.0b	1.1b
	Kale	13.4	85.2a	4.0b	1.0b
	Lettuce	12.0	32.0b	10.2a	1.9a
	Malabar spinach	-	81.2a	4.3b	1.2b
	P-value	NS	***	***	**

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference

test ($\alpha = 0.05$)

ⁱⁱNon-reported for the malabar

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 2.15. Average texture, vitamin C, antioxidant capacity, and total with different nutrient solution volumes low (75 L; 2 L per plant) and high (150 L; 4 L per plant) across arugula ‘Standard’, kale ‘Red Russian’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ in greenhouse settings during the summer and fall trials in the greenhouse

Season	Species	Volume (L)	Texture (N·g ⁻¹)	Vitamin C (mg·100 g ⁻¹ FW)	Antioxidant Capacity (μmol TE·100 g ⁻¹ DW)	Total Phenolics (mg GAE·100 g ⁻¹ DW)
Summer	Arugula	75	36.0	96.8	3.1a ⁱ	1.1
		150	36.2	70.7	3.3a	1.0
	Kale	75	75.0	69.0	5.1a	0.9
		150	67.2	58.3	3.0a	0.8
	Lettuce	75	19.7	25.3	6.7a	1.3
		150	15.2	29.2	2.7b	0.4
	Malabar spinach	75	- ⁱⁱ	70.4	3.6a	1.0
		150	-	72.6	2.8a	0.8
	Volume		NS ⁱⁱⁱ	NS	*	*
	Species		***	**	NS	NS
Volume×Species			NS	NS	NS	NS
Fall	Arugula	75	13.1	88.5	3.3	1.3
		150	13.4	113.3	2.6	1.0
	Kale	75	13.1	95.9	4.2	1.0
		150	13.8	74.4	3.8	1.1
	Lettuce	75	13.1	31.2	8.3	1.7
		150	10.9	32.9	12.1	2.1
	Malabar spinach	75	-	87.6	3.8	0.9
		150	-	74.8	4.8	1.5
	Volume		NS	NS	NS	NS
	Species		NS	***	***	**
Volume×Species			NS	NS	NS	NS

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$)

ⁱⁱNon-reported for the Malabar spinach

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001 respectively

2.4 Discussion

2.4.1 Estimated N Uptake Efficiency and N Retention with Different Nutrient

Solution Volumes

N uptake efficiency was influenced by NS volume. The higher N uptake efficiency observed in the low volume treatment at W4 during the summer trial and W2 during the fall trial suggests that lower NS volume may be advantageous at different growth stages, depending on the season. Despite these differences, N recovery in plant tissues remained consistent across

volumes, indicating similar total N utilization by the plants. However, the high volume treatment resulted in greater N retained in the NS reservoirs, reflecting more residual N in the system, which may lead to increased nutrient waste. These findings suggest that using a lower NS volume can enhance N uptake efficiency while reducing residual N, thereby promoting more sustainable hydroponic production.

2.4.2 Growth and Yield in Response to Nutrient Solution Volume

The NS volume did not significantly affect most growth parameters, including plant height, leaf number, SPAD value, shoot and root fresh weight, shoot dry weight, leaf area. The only exception was root dry weight in the fall trial. This seasonal variation may be attributed to cooler temperatures in the fall (average 20.7 °C), which could have prompted plants to allocate more resources to root growth in order to maximize nutrient uptake (Gowin et al., 1980). However, this result contrasts with Sublett et al. (2018) who used a volume of 10 L (1.6 L per plant) NS and a high EC of 4.5 mS·cm⁻¹. They observed higher root dry mass in lettuce in spring compared to summer, which may be attributed to the higher EC level used in their study compared to ours (1.8 mS·cm⁻¹).

In our research, no differences in shoot and root fresh weight were observed between low and high NS volume treatments. This finding differs from Miller et al. (2020), who reported reduced shoot fresh and dry weights and shoot water content when using recycled NS adjusted to an EC of 1.8 mS·cm⁻¹ three times per week with pH maintained at 6.2 to 7.1 compared to control (fresh NS). These reductions were linked to nutrient depletion, specifically of N, P, K, and Fe, along with Na accumulation in both plant tissues and the NS. The limited NS volume of 20 L (1.3 L per plant) may have contributed to these negative effects by limiting nutrient availability and buffering capacity. In contrast, our research used a larger NS volume of 2 L (low volume)

and 4 L (high volume) per plant, combined with continuous recirculation and daily adjustments to maintain EC at $1.8 \text{ mS}\cdot\text{cm}^{-1}$ and pH within 5.8 to 5.9. These practices likely contributed to more stable nutrient conditions and consistent plant growth across both volume treatments. Similar findings were supported by Bonasia et al. (2017), who reported a 18.7% reduction in fresh yield when EC reached $3.5 \text{ dS}\cdot\text{m}^{-1}$ under a NS volume of 50 L (2.5 L per plant) that was likely due to osmotic stress. The daily EC adjustment in our study may have helped to prevent such stress. This approach is supported by Currey et al. (2019), who emphasized the importance of daily EC regulation in maintaining a consistent nutrient supply.

Maintaining stable pH and EC levels contributes to consistent growth performance. Gillespie et al. (2021) applied four treatments to grow spinach in Deep Water Culture (DWC): low pH 4.5 with standard EC $1.4 \text{ dS}\cdot\text{m}^{-1}$ (LP/SE), low pH 4.5 with high EC $3.4 \text{ dS}\cdot\text{m}^{-1}$ (LP/HE), standard pH 5.5 with standard EC $1.4 \text{ dS}\cdot\text{m}^{-1}$ (SP/SE), and standard pH 5.5 with high EC $3.4 \text{ dS}\cdot\text{m}^{-1}$ (SP/HE). They observed that maintaining NS at LP/SE significantly reduced shoot and root fresh and dry weights, leaf number, and leaf area in spinach by 62%, 44%, 55%, 23%, 19% and 55%, respectively, compared to SP/SE. They also reported that SP/HE partially improved growth performance but did not fully restore growth to the level of SP/SE. These findings highlight the importance of maintaining optimal pH and EC in hydroponic systems, as adjusting EC alone cannot compensate for the negative effect of low pH on nutrient availability, and thus plant growth, in hydroponic systems.

A significant interaction between NS volume and species for shoot fresh weight was observed in the summer trial, indicating species-specific responses to volume treatments. Kale produced the highest shoot fresh weight under the high volume treatment compared to lettuce at the same volume and Malabar spinach under both volumes. However, no differences were

observed between kale and arugula in both volume treatments. Although the NS volume did not significantly affect shoot fresh weight within an individual species, shoot fresh weight numerically increased in kale and arugula and decreased in lettuce and Malabar spinach in high volume treatment. This result highlights that further research is needed to clearly understand species-specific responses to NS volume in a hydroponic system.

Additionally, a clear seasonal effect was observed in our research. All species produced higher shoot fresh weight during the summer trial, likely due to higher temperatures (averaged 24.5 °C) and increased light intensity, both of which enhance the photosynthetic efficiency and transpiration rates. These findings are consistent with those of Devadze et al. (2025), who emphasized the role of temperature, light intensity, CO₂, and relative humidity in regulating plant physiological responses. Similarly, Djidonou & Leskovar (2019) reported that warmer seasons had a greater influence on relative growth rate and yield.

2.4.3 Shoot Mineral Nutrient Concentration in Response to Nutrient Solution

Volume

When data were pooled across species, we found that NS volume influenced the shoot N, P, and K content, with effects varying by season. The high volume treatment resulted in greater N and K content during the summer trial, possibly due to more stable nutrient concentrations throughout the growing period, increased total N input, and enhanced metabolic activity at higher temperatures (Petropoulos et al., 2016). These conditions elevate plant demand for N and K, which are essential for photosynthetic activity and overall growth. Our findings align with those of Fallovo et al. (2009), who reported higher N and K concentrations in summer compared to spring under a 65 L NS volume (0.2 L per plant). In the fall trial, higher P and K

concentrations with the high volume treatment were likely due to more stable and sufficient nutrient availability, even under cooler conditions.

Shoot (including both leaves and stems) N, P, and K concentrations across species were compared with the leaf tissue sufficiency ranges reported by Bryson & Mills (2014) and Mattson & Merrill (2015). In arugula, shoot N concentration exceeded the sufficiency range in both summer and fall trials under both NS volumes. This aligns with findings by Yang et al. (2021), who reported that the highest N accumulation in arugula occurred at EC of 1.5 and 1.8 dS·m⁻¹. Lower or higher EC levels, such as EC 1.2 mS·cm⁻¹ and 2.1 mS·cm⁻¹ in their study, led to N deficiency and reduced chlorophyll content. Similarly, shoot P concentrations in arugula remained within or above the sufficiency range in both seasons in our research. Shoot K concentration in the high volume treatment during the summer and in both volume treatments during the fall exceeded the sufficiency range. In contrast, shoot K concentrations in the low volume treatment during the summer were 8% below the lower sufficiency threshold. This deficiency may be related to antagonistic interactions with Ca or Mg, as suggested by Meselmani (2022), although these elements were not measured in the present study.

In lettuce, shoot N, P and K concentrations remained within the sufficient ranges reported by Campbell (2000) in both the summer and fall trials. These results align with those of Samarakoon et al. (2019), who observed increased N and K uptake in lettuce with rising EC up to 1.8 mS·cm⁻¹, while P uptake remained stable. Our findings suggest that a low NS volume maintained at EC 1.8 mS·cm⁻¹ is adequate to support optimal nutrient uptake in lettuce.

In kale, shoot N, P, and K concentrations were above the sufficiency range under both NS volumes in the summer trial. In the fall trial, shoot N concentration fell within the sufficiency range, while shoot P and K concentrations remained above it.

Unlike other leafy green species, the sufficiency range of nutrients in Malabar spinach has not been defined. Although the plant appeared generally healthy in the current study, slight yellowing of some old and younger leaves in both volume treatments suggests possible interveinal chlorosis, which can be associated with an inadequate amount of N, Mg, Fe, and Mo (Croze, 2019). While Malabar spinach can tolerate a soil pH range of 4.3 to 7.5 (Fern, 2014), its pH tolerance under hydroponic production remains unverified. This reinforces the need for further research to optimize EC and pH for Malabar spinach production in a hydroponic system.

2.4.4 Health Implications of Lower K and Nitrate

In the current study, shoot K concentration varied among leafy green species, with Malabar spinach and lettuce showing the highest levels, while arugula and kale had comparatively lower concentrations. Although vegetables are generally rich in K (Renna et al., 2018; Weiner & Wingo, 1998), excessive K intake can pose health risks, particularly for individuals with chronic kidney disease (CKD) (National Kidney Foundation, 2023; Renna et al., 2018). CKD affects approximately 8-13% of the global population and is a growing public health concern (Delanaye et al., 2017). For CKD patients, impaired kidney function reduces the ability to excrete K, increasing the risk of hyperkalemia (Kes, 2001), which can cause slow heart rate, limb numbness, and, in severe cases, paralysis or cardiac arrest (Spital & Stems, 1988).

Based on our findings, arugula and kale may be suitable options for K-restricted diets due to their relatively low K content. Previous studies have similarly focused on producing low-K vegetables such as red leaf lettuce, asparagus lettuce, komatsuna (Japanese mustard spinach), tomatoes, onions, and melons (Asao et al., 2013; Ogawa et al., 2012; Okada et al., 2020; Tsukagoshi et al., 2016). They used different strategies such as reducing potassium nitrate concentration in NS, restricting K supply entirely or at stage growth stage, altering NS every

week with or without K, and evaluating mineral absorption patterns during growth and development. However, these strategies can be time-consuming and labor-intensive for growers. In contrast, our results suggest that managing NS volume may be a simpler alternative. Species such as arugula, kale, lettuce, and Malabar spinach can be successfully cultivated in hydroponic systems using low NS volume, particularly during the fall season, while still maintaining acceptable K levels.

Additionally, excessive nitrate concentration in food has been linked to several health risks, including cancers of the stomach, intestine, bladder, and mouth, as well as methemoglobinemia (commonly known as ‘blue baby syndrome’) in children (Salehzadeh et al., 2020). In the current study, observed nitrate concentrations were compared with the safety thresholds established by European Commission (EU) Regulations No 1258/2011 (Colla et al., 2018). In the present study, nitrate concentration was influenced by both NS volume and season. During the summer trial, lettuce grown under the high NS volume exceeded the nitrate safety threshold limit by 50%. In contrast, all other two species (arugula and lettuce) grown under the low volume treatment remained within the safe threshold limit during both seasons. However, kale consistently exceeded the safe threshold limit by 5-6 times under both NS volumes and in both seasons, highlighting its inherently high nitrate accumulation capacity. No nitrate safe range was available for Malabar spinach; however, when considering the safe range limit for lettuce as a reference, nitrate level in Malabar spinach remained within the safe range under low volume treatment in both seasons. These results suggest that reducing NS volume can help limit nitrate accumulation in leafy greens such as lettuce, arugula and Malabar spinach, thereby supporting safer hydroponic production practices

2.4.5 Color and Texture in Response to Nutrient Solution Volume

NS volume influenced color lightness (L^*), red/green value (a^*), and hue angle (h°), which reflect visual characteristics such as brightness, redness, and color tone. In the present study, increased h° and lower L^* values under the low volume treatment indicate a deeper green coloration in leafy greens, suggesting enhanced chlorophyll content or pigmentation density. A significant interaction between NS volume and species further demonstrates that color response is species-dependent. Notably, arugula and Malabar spinach displayed low L^* values under the low volume treatment, indicating a darker green appearance. These findings suggest that low NS volume supports the development of a dense green color in these species, which may be desirable from a consumer perspective.

In contrast, higher a^* values, representing redness, were observed in kale and lettuce under the high volume treatment, reflecting an increase in red pigmentation under these conditions. Similarly, h° value of 91.1 in lettuce and 91.4 in kale under high volume treatment fall within the range of yellow to yellow red. However, anthocyanin concentration in lettuce was numerically higher in the low volume treatment, despite the higher a^* values (redness) and h° of 91.1 (yellow to green) under high volume. This discrepancy suggests that visual redness does not solely correspond with anthocyanin content. Similar explanations were supported by Marin et al. (2015), who found that hue angle (h°) did not reflect reliable anthocyanin accumulation in red lettuce, likely due to the presence of chlorophyll, carotenoids and co-pigmenting phenolics that influence overall leaf color. To date, limited research has addressed the effect of NS volume on color indices in leafy greens. The findings of this study provide new insight into how NS volume influences visual quality indices, which are important indicators of consumer perception and marketability.

NS volume did not significantly affect texture or firmness in the present study. This lack of response may be related to inherently higher lignin content in the cell walls of hydroponically grown crops, as reported by Lei & Engeseth (2021) in lettuce. Previous studies on lettuce have shown inconsistent relationships between nitrogen concentration and texture. For example, Cuppett et al. (1999) reported that increasing N concentration from 30 to 120 mg·L⁻¹ improved firmness, while Gutiérrez-Rodríguez et al. (2013) observed a negative correlation between N and texture. Although NS volume had no effect, species-specific differences in texture were evident. Kale and arugula had a firmer texture compared to lettuce and Malabar spinach. In contrast, softer texture was observed in lettuce. These findings contrast with the results of Onge (2014), who reported that softer textures in lettuce, arugula, and kale compared to true spinach. This discrepancy is possibly due to cultivar differences, which were not specified in that study. These findings were supported by Barker et al. (2022), who reported variation in crispiness and hardness among different kale cultivars.

2.4.6 Total Phenolics, Antioxidant capacity, and Vitamin C in Response to Nutrient Solution Volume

Based on the pooled data analysis across all species, NS volume influenced total phenolics and antioxidant capacity in a season-dependent manner, while having no significant effect on anthocyanin or vitamin C concentrations. In the summer trial, the low volume treatment resulted in higher total phenolics and antioxidant capacity. However during the fall trial, NS volume did not impact any phytochemical concentrations.

The seasonal effect for total phenolics and antioxidant capacity may be because of higher light intensity in the summer season, as supported by (Colonna et al., 2016), who reported elevated total phenolics in chicory, mizuna and Swiss chard under high light intensity in

greenhouse conditions. Total phenolics and antioxidant capacity were numerically higher in the fall compared to summer by 9% and 6.5%, respectively, in the low volume treatment and by 100% for both parameters in the high volume treatment. This corresponds with the findings of Sublett et al. (2018) who reported higher phenolic content in lettuce during the fall and spring season compared to summer. However, our results differ from those reported by Bunning et al. (2010), who observed no seasonal variation in total phenolics or antioxidant capacity in lettuce, likely due to their field-based production system. Similarly, Differences in cropping systems can significantly affect plant secondary metabolism (Weston & Barth, 1997). In the fall trial, total phenolics and antioxidant capacity varied among species, with lettuce showing higher concentrations than other tested leafy greens. This finding aligns with Liu et al. (2007), who reported higher total phenolics in lettuce, attributed to its higher anthocyanin contents and stronger antioxidant capacity of anthocyanins.

These results highlight that species selection is a strong determinant of total phenolics and antioxidant capacity, as also noted by Nassar et al. (2015). Interestingly, in both summer and fall trials, arugula, Malabar spinach and kale had higher vitamin C concentrations than lettuce. These species may offer greater nutritional value, as vitamin C is a potent antioxidant that scavenges reactive oxygen species such as hydroxyl and superoxide anion radicals, which are associated with cancer development (Ishikawa & Shigeoka, 2008). In the present study, arugula contained twice the vitamin C concentration reported for commercially grown arugula by Xiao et al. (2012). Kale showed vitamin C concentrations of 63.45 mg·100 g⁻¹ FW in the summer and 85.15 mg·100 g⁻¹ FW in the fall, consistent with the range provided by Satheesh & Workneh Fanta (2020). These values also meet the daily recommended intake for both males and females, which ranges from 62.27 mg·100 g⁻¹ to 969 mg·100 g⁻¹ FW (Satheesh and Workneh Fanta,

2020). Vitamin C concentration in lettuce has been reported to vary widely, from 28 mg·100 g⁻¹ FW to 220 mg·100 g⁻¹ FW, depending on the cultivar and variety (Kim et al., 2016). In our study, vitamin C levels in lettuce were more than twenty-six times in the summer and thirty-two times in the fall compared to hydroponically grown lettuce reported by Buchanan & Omaye (2013). Additionally, the higher antioxidant capacity observed in lettuce under the low volume treatment further supports the potential of this approach to enhance the nutritional quality of leafy greens.

2.5 Conclusion

Our research indicated that using a low NS volume of 75 L (2 L per plant) resulted in higher N uptake efficiency and lower N retention in an NFT hydroponic system in both summer and fall trials. While NS volume had no significant impact on overall growth and yield parameters with daily management of NS pH and EC, it influenced nutrient composition and quality attributes of leafy greens. Specifically, high NS volume of 150 L (4 L per plant) increased shoot N and K concentrations in the summer and P and K in the fall. Shoot N, P and K concentrations in arugula, kale and lettuce were maintained within or above the sufficiency range. Notably, lower shoot K concentrations in arugula and kale may offer a potential dietary benefit for individuals with chronic kidney disease. Low NS volume resulted in safe shoot nitrate levels in arugula and lettuce across both seasons, while kale consistently exceeded safety thresholds regardless of treatment. NS volume also influenced visual quality indices, as reflected by changes in color indices, and contributed to enhanced nutritional quality. In particular, antioxidant capacity and total phenolic concentration were higher under low volume in the summer, potentially due to higher light intensity. Overall, these findings suggest that a NS of 75 L (2 L per plant), combined with daily maintenance of EC and pH within optimal ranges, optimized yield, visual quality and nutritional value of hydroponically grown arugula and lettuce

in a recirculating hydroponic system while improving nutrient uptake efficiency. While kale produced the highest shoot fresh weight under these conditions, it also accumulated the highest nitrate levels beyond the safety threshold limit, which suggests a food safety concern. To our knowledge, this is the first report that evaluates Malabar spinach in a hydroponic system, and based on foliage color, a higher EC than the standard $1.8 \text{ mS}\cdot\text{cm}^{-1}$ may be necessary to optimize growth. However, further research is needed to establish the nutrient sufficiency ranges and safe nitrate limits of this crop.

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Chapter 3 - Nutrient Solution Electrical Conductivity Effects on Growth and Quality of Four Leafy Greens and Herb Species Produced with Recirculating Hydroponics in Indoor Growth Tents

Abstract

Electrical Conductivity (EC) is widely used as a metric to manage recirculating nutrient solution (NS) in hydroponic systems. However, comprehensive research regarding optimal EC levels for many leafy green vegetables and herbs in hydroponic cultivation remains limited. An experiment was conducted using two NS EC levels (standard: 1.8 and elevated: 3.6 mS·cm⁻¹) with four plant species: basil (*Ocimum basilicum* ‘Large Leaf’), kale (*Brassica oleracea* ‘Winterbor F1’), butterhead lettuce (*Lactuca sativa* ‘Salanova Red Butter’), and the emerging crop Malabar spinach (*Basella alba* ‘Rubra’) with vertical nutrient film technique (NFT) hydroponics in an indoor lab using an indoor growth tent. Results showed that plant height and SPAD values were higher with elevated EC across species, with SPAD particularly increased in Malabar spinach. Shoot fresh weight across species was increased by elevated EC across all species. Shoot and root dry weight were increased by elevated EC, particularly in kale. Tissue analyses revealed high potassium (K) concentration at elevated EC. Standard EC reduced nitrate levels in basil, lettuce and Malabar spinach to within safe range limits, while kale surpassed recommended safe nitrate levels regardless of treatment. Standard EC levels could be a potential strategy to produce K-limited leafy greens to support a low K diet for kidney-diseased patients. Postharvest quality, including color, texture, and nutritional quality, vitamin C, anthocyanin, total phenolics, and antioxidant capacity, was not affected by EC levels. Based on our results, standard EC is optimal for basil and lettuce, while elevated EC may benefit kale and Malabar spinach. For Malabar

spinach, high wire production systems should be explored to support its vining growth habit, extend harvest duration to increase yield, and enhance the optimal foliage color under higher EC levels. These findings highlight the importance of crop-specific NS management to optimize plant health and nutritional quality.

Keywords: antioxidants, basil, kale, lettuce, Malabar spinach, vertical hydroponic system, nutrient film technique, nutrient management, nitrate, phenolics, vitamin C

3.1 Introduction

Sustainable agricultural production in urban areas is severely hampered by the expanding urban population and the decreased quantity of arable land available (Nwosisi & Nandwani, 2018). Controlled environment agriculture (CEA) can offer a potential solution by increasing production efficiency, maximizing yields, and improving crop quality through year-round production in an optimized environment (Gómez et al., 2019; Jensen, 2002). Hydroponics, a key production system within CEA, is particularly relevant in urban agriculture. More than half of the CEA producers use hydroponics solely (Walters et al., 2020). Over 80% of hydroponic growers monitor air temperature, nutrient solution (NS), pH, and electrical conductivity (EC) to optimize the growth conditions of the plants, while 56% of them monitor the intensity of light (Walters et al., 2020). This suggests that, for hydroponic systems, close monitoring of the environmental and nutrient parameters is crucial for optimizing the growth and quality of crops.

Nutrient management is essential to deliver optimal levels of nutrients, water and oxygen uniformly throughout the growth period (Niu & Masabni, 2022). A key parameter of growth optimization in a hydroponic system is nutrient level. Commercial growers widely use EC as a standard indicator to manage nutrient levels (Samarakoon et al., 2020). EC represents the concentration of dissolved salts and ions and determines fertilizer concentrations present in the root zone, which ultimately determines the strength of the NS (Gorbe & Calatayud, 2010). EC directly impacts plant development, yield, and nutritional quality (Tzortzakis et al., 2020) and directly influences phytochemical content, nutritional value, and aesthetic appeal of crops (Yang et al., 2021). However, too low EC and high EC can indicate nutrient deficiencies or osmotic stress (Fathidarehnijeh et al., 2024). While EC typically ranges from 1.5 to 2.5 dS·m⁻¹, the optimum EC depends on the crop species (Asao, 2012).

Leafy greens have short growth cycles and high market demand, making them well-suited for hydroponic systems. It plays an indispensable role in human diets for their nutritional profile, coupled with health benefits. These crops are rich in vitamins A, C, and K; they support eyesight and maintain good skin health (Kumar et al., 2020). Lettuce has low calories, fat, and sodium (Na) but is rich in vitamins, folate, and fiber, as well as minerals including phosphorus (P), calcium (Ca), magnesium (Mg), and iron (Fe) (Kim et al., 2016; Medina-Lozano et al., 2021). It is also rich in phytochemicals such as ascorbic acid, carotenoids and tocopherols. Basil, an important herb species, is valued for its essential oils and compounds such as polyphenols, phenolics, and flavonoids, and its leaves have been used to treat a range of ailments, including fevers, coughs, flu, asthma, bronchitis, influenza, and diarrhea (Shahrajabian et al., 2020). Kale is one of the top 15 powerhouse vegetables and is known for its fiber, higher bioavailability of Ca compared to milk, prebiotic carbohydrates, and unsaturated fatty acids (Parkell et al., 2016; Satheesh & Workneh Fanta, 2020). Malabar spinach, commonly known as vine spinach, Ceylon spinach, Indian spinach and climbing spinach (Katiyar & Kumar, 2021). It is a heat-tolerant leafy green widely consumed in Asia and was successfully cultivated in tropical regions such as Hawaii and Puerto Rico (Qiu & Liu, 2020). While it is not true spinach, it shares several benefits for instance, it is rich in vitamin A and C, carotenoids, phenols, alkaloids, tannins, bioflavonoids, organic acid, betacyanin, and minerals such as Ca, P, potassium (K) and Mg (Qiu & Liu, 2020). With increasing consumer demand for diverse leafy green varieties, Malabar spinach offers expanded market potential in urban areas, especially with restaurants and grocers specializing in seasonal, ethnic and local products (Ernst, 2017; Parkell et al., 2016). However, the species' production has not been extensively studied in hydroponic systems.

Although EC-based nutrient management is highly popular for commercial hydroponic growers, there is limited research on EC optimization for less-studied leafy greens and herbs, such as kale and Malabar spinach, using hydroponic systems. Consequently, growers often follow the guidelines developed for lettuce, one of the most cultivated hydroponic crops (Yang et al., 2024). However, applying the lettuce-based guideline to other leafy greens and herb species with different nutrient uptake patterns can limit their growth and quality. To address this gap, our study aims to explore how different levels of EC affect the growth and quality of several potential leafy green vegetables in growth tent facilities using the recirculating hydroponic system. This research will provide insights to improve nutrient management techniques for diverse leafy greens and herb species, supporting more efficient and sustainable hydroponic production.

3.2 Materials and Methods

3.2.1 Experimental Overview and Plant Materials

Experiments were conducted at Kansas State University Olathe in an indoor lab from August to October 2024 using an indoor growth tent (VIVOSUN, Ontario, CA, USA). The experiment followed a split-plot experimental design, with the electrical conductivity (EC) of NS as the main plot and plant species as subplots. Two EC of NS were standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) were used in the study. The four plant species were basil (*Ocimum basilicum* ‘Large leaf’), kale (*Brassica oleracea* ‘Winterbor F1’), butterhead lettuce (*Lactuca sativa* ‘Salanova Red Butter’) and Malabar spinach (*Basella alba* ‘Rubra’). The experiment was repeated twice in August to September (Trial 1) and September to October (Trial 2).

Seeds of the four plant species (Johnny’s Selected Seeds, Albion, ME, USA) were sown in square agrifoam (Horticubes®; Smithers Oasis, Canton, OH, USA) using a small nutrient film

technique (NFT) desktop system (53.3cm L × 33.0 cm W × 15.2 cm H; CropKing, Lodi, OH, USA) under a photoperiod of 16 hours and a daily light integral (DLI) of 10 mol m⁻²·day⁻¹ and LED grow lights (Model 10101-10135A; Root Farm, Marysville, OH, USA). Healthy, uniform, and robust seedlings were transplanted 14 to 15 days after sowing. Transplanting was done into a horizontal stacked vertical recirculating hydroponic system based on the nutrient film technique (NFT) in an indoor growth tent with an area of 4.6 m² (304.8 cm L × 152.4 cm W × 203.2 cm).

The main vertical hydroponic NFT system (94.99 cm L x 66.04 cm W x 97.03 cm H; Model VVGrey4-36; VEVOR, Bloomington, CA, USA), which consists of four horizontally oriented growth pipes, was used in this study. Each pipe contained 9 square planting holes spaced 5.08 cm apart, resulting in 36 plant spaces in each system. To include the uniform number of plants of four species in each pipe, one hole had been sealed, resulting in 32 plants per system (8 plants per species). Three systems were used for each of the EC treatments. A 8-gallon (30-L) reservoir tank (Artisun Technology LLC, Southport, CT, USA) was equipped with a circulation pump rated at 0.88 gpm (VEVOR, Bloomington, CA, USA) to deliver 20 L of NS to the growth pipe.

The growth tent consists of a smart air filtration system (PRO S8 Kit; VIVOSUN, Ontario, CA, w ECUSA), a clip-on fan (Aero Wave E6; VIVOSUN), and a 100 watts tunable spectrum LED grow light (Aerolight A100; VIVOSUN) with an integrated circulation fan. The environmental conditions, such as temperature, humidity, and light intensity, were controlled through the controller (VIVOSUN). The set conditions were set to an average day temperature of 21.3°C and night temperature of 18°C, average relative humidity of 62 %, photoperiod of 16 hours light and 8 hours dark, daily light integral (DLI) of 14.4 mol·m⁻²·d⁻¹ and photosynthetic photon flux density (PPFD) of 230 μmol·m⁻²·s⁻¹.

3.2.2 Nutrient Solution Preparation

Fertilizer stock solution was prepared separately using a two-part water-soluble fertilizer (Hydro Grow Leafy Green; CropKing, Lodi, OH, USA) at $97.5 \text{ g}\cdot\text{L}^{-1}$ and calcium nitrate (CropKing, Lodi, OH, USA) at $75 \text{ g}\cdot\text{L}^{-1}$ as shown in Table 3.1.

Table 3.1. Composition of nutrients and their concentration in two-part fertilizer of leafy green vegetables

Nutrients	Composition	Concentration
Hydro-Gro Leafy Greens (Part A)		
Macronutrients	Total Nitrogen (N)	4.3 %
	Available Phosphoric acid (P_2O_5)	9.3 %
	Soluble Potash (K_2O)	35 %
	Mg	3.9 %
Trace elements	Boron (B)	$200 \text{ mg}\cdot\text{L}^{-1}$
	Copper (Cu)	$105 \text{ mg}\cdot\text{L}^{-1}$
	Fe	$2100 \text{ mg}\cdot\text{L}^{-1}$
	Manganese (Mn)	$190 \text{ mg}\cdot\text{L}^{-1}$
	Molybdenum (Mo)	$42 \text{ mg}\cdot\text{L}^{-1}$
	Zinc (Zn)	$210 \text{ mg}\cdot\text{L}^{-1}$
Calcium Nitrate $\text{Ca}(\text{NO}_3)_2$ (Part B)		
Macronutrients	Total N	15.5 %
	Ca	19 %

To prepare the desired EC levels, the reservoir was initially filled with 75% of municipal water (EC: $0.37 \text{ mS}\cdot\text{cm}^{-1}$; pH: 7.1; total alkalinity: $95 \text{ mg}\cdot\text{l}^{-1}$), followed by the addition of equal volume of stock solutions A and B to achieve target EC values for the standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) treatments, within a standard error of ± 0.05 . The total NS volume was maintained at 20 L and the pH was kept within the range of 5.8-5.9. The NS volume, EC, and pH were monitored three times per week using an EC and pH meter (HANNA[®] instruments,

Smithfield, RI, USA). pH was adjusted daily using 10% H₂SO₄ (prepared from 25% concentrated sulfuric acid) to decrease pH, and 5% KOH (potassium hydroxide) to increase pH.

3.2.3 Measurement of Plant Height, Number of Leaves, and SPAD

Plant height, leaf number, and SPAD value were measured biweekly on all eight plants per species as an experimental unit. Plant height was determined by recording the distance from the base of the stem to the top of the foliage canopy. The number of true leaves was counted for each plant. SPAD value (an index of chlorophyll content per unit leaf area) for each plant was observed by measuring 3 to 4 fully expanded leaves at the central point between midrib and leaf margin and recorded using a handheld soil-plant analysis development (SPAD) meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan).

3.2.4 Harvest and Measurement of Yield Parameters

All the yield parameters (shoot and root fresh weight, shoot and root dry weight, shoot and root water content, and percent shoot dry matter and root dry matter) were measured on all eight plants per species as an experimental unit. In both trials, leafy green vegetable species used in the experiment were harvested 27 days after transplanting. The shoot and root fresh weight were recorded using a precision scale (OHAUS, Parsippany, NJ, USA). Leaf area measurements were conducted on the day of harvest by assessing each leaf from every plant using a leaf area meter (LI-3100C; LI-COR Environmental, Lincoln, NE, USA). Subsequently, shoot and root samples from each plant were promptly bagged in brown paper bags and oven-dried at 75 °C until constant weight. Then shoot and root dry weight was recorded. The shoot and root water

content were calculated using Equation 3.1, and the percent shoot and root dry matter content were calculated using Equation 3.2.

Equation 3.1. The percentage of shoot and root water content

$$\frac{(FW-DW)}{FW} \times 100$$

Equation 3.2. The percentage of shoot and root dry matter content

$$\frac{DW}{FW} \times 100$$

where FW: fresh weight of either shoot or root, DW: dry weight of either shoot or root

3.2.5 Measurement of Nutrients in Plant Tissue

All dried shoot samples (both leaves and stems) harvested in the end of the experiment were ground using a mill (Cyclotec™ 1093; FOSS Analytical, Hillerød, Denmark) and sent to the Soil Testing Laboratory for analysis of total N, P, K, and nitrate concentration. Total N, P, and K in plant tissue were analyzed using the sulfuric peroxide digestion method (Lindner & Harley, 1942; Thomas et al., 1967). The resulting digest, which contains ammonia, was analyzed by an indophenol blue colorimetric procedure using the Rapid Flow Analyzer (Model RFA-300) from Alpchem Corporation (Clackamas, OR, USA), following RFA Methodology No. A303-S072. P and K concentrations were determined using an Inductively Coupled Plasma (ICP) Spectrometer meter (Model 5800 ICP OES; Agilent Technologies, Santa Clara, CA, USA).

3.2.6 Foliage Color and Texture

For the postharvest analysis, fresh shoot samples were harvested and transported to the Postharvest Physiology lab at K-State Olathe. Surface color was determined based on three plant samples per species for each treatment replication, using four outer leaves per plant. Measurements were taken with a chromameter (A5 CR-400; Minolta Co.Ltd., Osaka, Japan) and expressed in the International Commission on Illumination (CIE) L*a*b* color space, where L*

indicates lightness, a^* denotes the red/green value and b represents yellow to hue (Bonasia et al., 2017). Hue angle was expressed on a 360° color wheel where 0° and 360° represent red, 270° represents blue, 180° represents green, and 90° represents yellow. Texture was measured on three plant samples per species for each treatment replication. Five outer leaves without midrib from each replication for lettuce, three outer leaves with midrib from each replication for basil, and three outer leaves without midrib from each replication for kale were determined for texture. The texture was not performed for Malabar spinach because of an inadequate number of leaves. Textures were measured using a texture analyzer (TA-58, TA.XT.plus, Texture Technologies Corp., Scarsdale, NY, USA) with a Kramer Shear Cell (TA-91; Texture Technologies Corp., Scarsdale, NY, USA) equipped with five blades. Leaf texture was calculated as the maximum force in Newton per gram (Batziakas et al., 2020).

3.2.7 Nutritional Quality

Nutritional quality, including phytochemicals and vitamin C was determined by using three fresh plant shoot samples per species (basil, kale, lettuce and Malabar spinach) for each treatment replication. Plant samples were cut into pieces with a knife and homogenized. For each treatment replication, two samples of 40 grams of fresh leaves per species were collected for anthocyanin, total phenolics and antioxidant capacity. The collected samples were frozen and stored at -20°C for further analysis. The frozen samples were freeze-dried using a freeze dryer (Harvest Right, North Salt Lake, UT, USA). All samples were finely pulverized into powder using a pestle and mortar in liquid N and transferred into centrifuge tubes for the following analysis.

3.2.7.1 Phytochemicals

Anthocyanin content was measured only in lettuce, as red butter lettuce has a vibrant red color rich in anthocyanin concentration (Anum et al., 2024). It was measured following the procedure provided by Adamse et al. (1989), Gazula et al. (2007), and Kleinhenz et al. (2003). Stored lettuce samples were immediately frozen in liquid nitrogen after harvest. Approximately 0.2 g of powder was transferred to a 15 ml centrifuge tube and extracted using 10 ml of methanol solution. The mixture was vortexed for 10 seconds and kept overnight at -20°C. On the following day, extracted samples were vortexed for 10 seconds and transferred into 1.75 ml tube and subsequently centrifuged (JA-17; Beckman Coulter, Palo Alto, CA, USA) for 10 minutes at 4°C at 8,000 rpm. The extract was diluted with 1% methanol. A final volume of 800 µl per diluted sample was prepared and vortexed. A volume of 250 µl from each sample and standard solution was plated in triplicate on a 96-well microplate. The absorbance was measured at 550 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Anthocyanin concentrations were calculated by using a standard curve generated with cyanidin-3-glucoside (C₃G) in 1% acidified methanol and were expressed as mg C₃G·100 g⁻¹ DW.

Total phenolics was measured for all four plant species by following the procedure described by Singleton and Rossi (1965). Stored plant samples were immediately frozen in liquid nitrogen after harvest. Approximately 0.2 g of powder was transferred to a 15 ml centrifuge tube and extracted using 10 ml of acetone and deionized water (v:v=1:1). The mixture was vortexed for 10 seconds and kept overnight at -20°C. On the following day, extracted samples were vortexed for 10 seconds and 100 µl was transferred into 1.75 ml tube. Subsample was diluted based on species using acetone and deionized water (v:v=1:1) for a final volume of 100 µl. Then, 500 µl of dilute Folin-Ciocalteu reagent was added to each sub-sample and standard solutions,

followed by 400 μl of sodium carbonate after 0.5-8 min. Subsamples were vortexed and incubated in a dryer at 30 $^{\circ}\text{C}$ for one hour. Then, it was centrifuged at 3,000 rpm for 5 min at 4 $^{\circ}\text{C}$. A 250 μl of each sample and standard solutions was plated in triplicate on a 96-well microplate. The absorbance was measured at 760 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Total phenolics were calculated by using a standard curve generated with gallic acid equivalents (GAE) and were expressed as mg GAE $\cdot$ 100 g $^{-1}$ DW.

Antioxidant capacity was analyzed for all four species using FRAP (Ferric Reducing Ability of Plasma) methods following the procedures described by Benzie & Strain (1996) and Benzie & Szeto (1999). The same extraction procedure used for total phenolics was followed. On the analysis day, extracted samples were vortexed for 10 seconds and transferred into a 1.75 ml tube. It was centrifuged at 11,300 rpm for 20 min at 4 $^{\circ}\text{C}$. Centrifuged extract was diluted based on the species in acetone and deionized water (v:v=1:1). For the assay, 200 μl of sample extract was diluted to 720 μl of fresh work reagent (10 mM TPTZ: Tripyridyltriazine, 20 mM FeCl_3 : Ferric chloride, and 300 mM sodium acetate buffer (pH 3.6) in a 1:1:10 ratio). A 230 μl of each sample and standard solutions were plated in triplicate on a 96-well microplate. The absorbance was measured at 593 nm using a 96-well microplate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA). Antioxidant capacity were calculated by using a standard curve and was expressed as $\mu\text{mol TE (Trolox equivalents)}\cdot$ 100 g $^{-1}$ DW.

Vitamin C, which is also known as ascorbic acid (AA), was measured all four plant species by following the procedure described by Klimczak & Gliszczynska-Swiglo (2015) and Desai & Desai (2019). A 2 g of homogenized samples from three plant samples per species per treatment were collected and mixed with 20 ml of acid solution (2 N glacial acetic acid and 6%

metaphosphoric acid) using a benchtop homogenizer (POLYTRON PT 1600 E; Kinematica Inc., Bohemia, NY, USA). The samples were stored at -20°C for further analysis. On the analysis day, samples were thawed and centrifuged at 12,000 rpm for 20 min at 4°C and again centrifuged for clarification. A 1 ml of clarified extract was used for the analysis of ascorbic acid (AA). To oxidize Ascorbic Acid (AA) to dehydroascorbic acid (DHAA), a drop of 0.2% of 2,6-dichloroindophenol (2,6-DCIP) solution was added, followed by incubation for 1 hour at room temperature. A 2% thiourea solution was added to precipitate the excess 2,6-DCIP, and then 2% 2,4-dinitrophenylhydrazine (2,4-DNPH) solution was added. Samples and standard solutions were incubated at 60°C for 3 hours in an oven (Heratherm™ Convection, ThermoFisher Scientific Inc., Asheville, NC, US) to allow the formation of ozone. After incubation, samples and standard solutions were cooled in an ice bath and reacted with 90% sulfuric acid. Absorbance was measured at 540 nm using a plate reader (Synergy H1; BioTek Instruments, Inc. Winooski, VT, USA) by following the procedure described by Terada et al. (1978). Vitamin C was quantified using a standard calibration curve and expressed as $\text{mg} \cdot 100 \text{ g}^{-1} \text{ FW}$.

3.2.8 Statistical Analysis

Statistical analysis was performed using software (SAS version 9.4, Cary, NC, USA). All data were pooled across trials as both trials were conducted under the same environmental conditions. A split-plot model was fitted through PROC GLIMMIX for each of the responses. The two levels of EC were the main plots. The four levels of species were subplots. Both the main effects and interaction effects were evaluated using Type III F-tests. Further, comparisons were carried out using LSMEANS. A two-sided t-test at the level of 0.05 with Tukey's adjustment was applied to the pairwise comparisons. Additionally, for the growth data, the week variable reflected

the time effect. Both the main effects and interaction effects were evaluated using Type III F-tests and further sliced by the time variable.

3.3 Results

3.3.1 Plant Height, Leaf Number, and SPAD in different EC levels

Pooled data across species were analyzed to evaluate the main effect of EC on plant height, leaf number and SPAD at week 2 (W2) and week 4 (W4) (Figure 3.2). A significant effect of EC was observed for plant height at W4 ($P<0.001$) and for SPAD at both W2 and W4 ($P<0.01$). Plant height increased by 14% at W4 and SPAD values increased by 7% and 6% at W2 and W4, respectively, at elevated EC compared to standard EC.

Figure 3.2 presents the effect of EC on plant height, leaf number, and SPAD of basil, kale, lettuce, and Malabar spinach at W2 and W4. There was no significant difference between the EC levels for plant height and leaf number in any species. However, EC had a significant effect on SPAD in Malabar spinach, where the SPAD at elevated EC treatment increased by 59% and 54% at W2 and W4, respectively, compared to standard EC. In contrast, the SPAD of the remaining species was unaffected by EC levels.

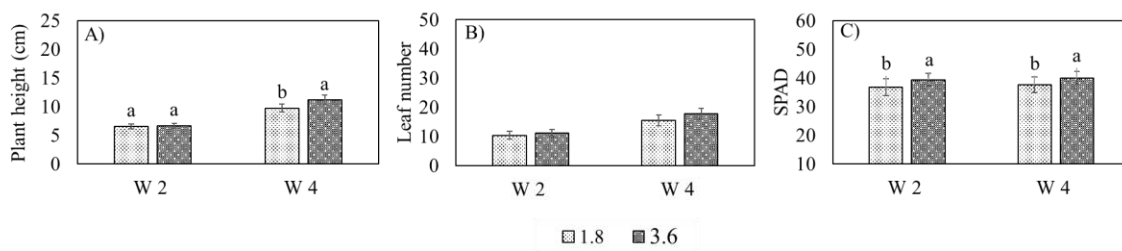


Figure 3.1. Average plant height (A), leaf number (B), and SPAD (C) across species with standard ($1.8 \text{ mS} \cdot \text{cm}^{-1}$) and elevated ($3.6 \text{ mS} \cdot \text{cm}^{-1}$) EC levels and across week 2 (W2) and week (W4) in an indoor growth tent throughout the study period

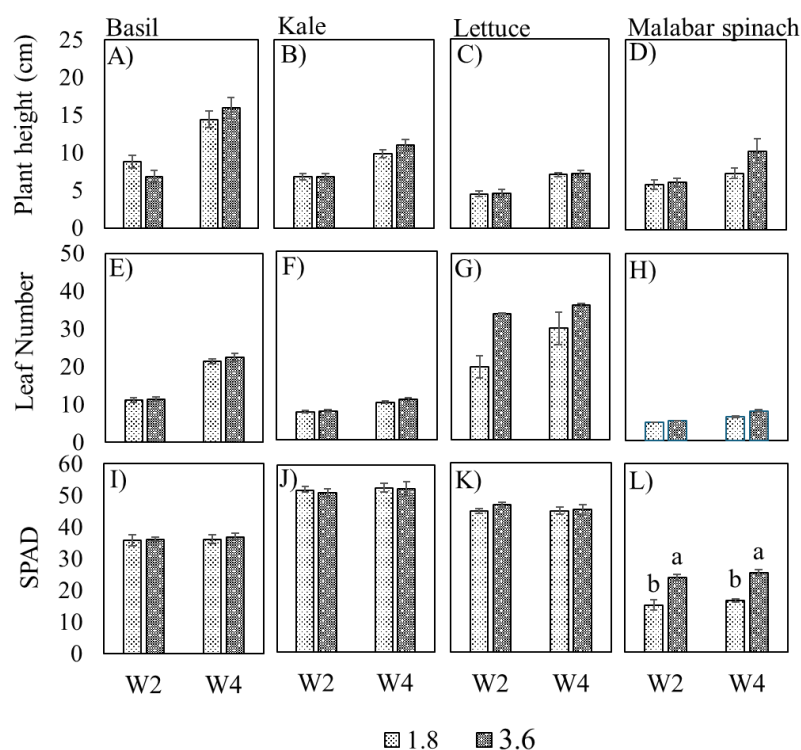


Figure 3.2. Average plant height (A, B, C and D), leaf number (E, F, G and H) and SPAD (I, J, K and L) with standard (1.8 mS·cm⁻¹) and elevated (3.6 mS·cm⁻¹) EC levels across basil ‘Larg Leaf’, kale ‘Winterbor F1’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ at week 2 (W2) and week 4 (W4) in an indoor growth tent throughout the study period

3.3.2 Plant Fresh and Dry Biomass, Leaf Area with Different EC levels

Pooled data across species were analyzed to evaluate the main effect of EC on the shoot and root fresh weight, shoot-to-root fresh weight ratio, shoot and root dry weight, and leaf area (Table 3.2). A significant difference between EC levels was observed for shoot fresh weight and shoot-to-root fresh weight ratio ($P < 0.05$), where shoot fresh weight and shoot-to-root fresh weight ratio at elevated EC increased by 11% and 8 %, respectively, compared to standard EC. However, root fresh weight, shoot and root dry weight, and leaf area were not affected by EC levels.

Table 3.3 presents the effect of EC, species and their interactions on shoot and root fresh weight, shoot-to-root fresh weight ratio, shoot and root dry weight, and leaf area. A significant

interaction between species and EC was found for the shoot-to-root fresh weight ratio ($P < 0.01$), and for shoot and root dry weight ($P < 0.05$). The shoot-to-root fresh weight ratio, particularly in Malabar spinach, increased by 41% at elevated EC compared to standard EC. The shoot and root dry weight, particularly in kale, increased by 21% and 57%, respectively, at elevated EC compared to standard EC. However, there was no interaction effect for shoot and root fresh weight, and leaf area for any other species.

Table 3.2. Average shoot fresh weight (SFW), root fresh weight (RFW), shoot to root fresh weight ratio (S:R), shoot dry weight (SDW), and root dry weight (RDW) across species with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

EC ($\text{mS}\cdot\text{cm}^{-1}$)	SFW (g)	RFW (g)	S:R Ratio	SDW (g)	RDW (g)	Leaf area (cm^2)
1.8	33.2b	9.4	3.8	1.7	0.5	487.8
3.6	37.0a	9.8	4.1	1.6	0.5	477.9
P-value	*	NS	*	NS	NS	NS

NS, *, ** mean non-significant or significant at $p \leq 0.05$, 0.01 , respectively

Table 3.3. Average shoot fresh weight (SFW), root fresh weight (RFW), shoot to root fresh weight ratio (S:R), shoot dry weight (SDW), and root dry weight (RDW) with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels across basil ‘Large Leaf’, kale ‘Winterbor F1’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ in an indoor growth tent throughout the study period

Species	EC ($\text{mS}\cdot\text{cm}^{-1}$)	SFW (g)	RFW (g)	(S:R) Ratio	SDW (g)	RDW (g)	Leaf Area (cm^2)
Basil (n=8) ⁱⁱ	1.8	23.6	9.9	3.0b	1.3a	0.5a ⁱ	351.5
	3.6	23.7	10.1	2.7bc	1.1a	0.4a	329.8
Kale (n=8)	1.8	47.3	11.1	4.9a	2.9b	0.7b	550.8
	3.6	55.3	12.1	5.0a	3.5a	1.1a	608.3
Lettuce (n=8)	1.8	47.9	9.7	5.2a	1.7a	0.4a	911.5
	3.6	47.2	9.2	5.4a	1.8a	0.4a	798.2
Malabar spinach (n=8)	1.8	14.0	6.9	2.2c	0.6a	0.3a	137.4
	3.6	21.8	7.8	3.1b	0.6a	0.3a	175.5
EC		* ⁱⁱⁱ	NS	*	NS	NS	NS
Species		***	***	***	***	***	***
EC $\times$ Species		NS	NS	**	*	*	NS

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$)

ⁱⁱn= total number of samples used per treatment of the reported average

ⁱⁱⁱNS, *, **, *** mean significant or significant at $p \leq 0.05$, 0.01 or 0.001 , respectively

3.3.3 Plant Water Content and Dry Matter Content with Different EC levels

Pooled data across species were analyzed to evaluate the main effect of EC on shoot and root water content and percent shoot and root dry matter content (Table 3.4). No significant difference between EC levels was observed for any of the parameters ($P>0.05$).

Table 3.5 presents the effect of EC, species and their interactions on shoot and root water content and percent shoot and root dry matter content. No significant interaction effect was observed for any of the above parameters ($P>0.05$).

Table 3.4. Average shoot water content (SWC), root water content (RWC) and shoot dry matter content (SDM), and root dry matter content (RDM) across species with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

EC ($\text{mS}\cdot\text{cm}^{-1}$)	SWC (%)	RWC (%)	SDM (%)	RDM (%)
1.8	95.1	94.4	4.9	5.6
3.6	95.3	94.3	4.7	5.8
P-value	NS	NS	NS	NS

NS, *, ** mean non-significant or significant at $p \leq 0.05$, 0.01 , respectively

Table 3.5. Average shoot water content (SWC), root water content (RWC) and percentage shoot dry matter content (SDM), and root dry matter content (RDM) with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels across basil ‘Large Leaf’, kale ‘Winterbor F1’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ in an indoor growth tent throughout the study period

Species	EC ($\text{mS}\cdot\text{cm}^{-1}$)	SWC (%)	RWC (%)	SDM (%)	RDM (%)
Basil (n=8) ⁱ	1.8	94.4	94.5	5.6	5.5
	3.6	94.9	95.9	5.1	4.1
Kale (n=8)	1.8	93.1	91.9	6.9	8.1
	3.6	93.2	90.1	6.8	9.9
Lettuce (n=8)	1.8	96.4	95.8	3.6	4.3
	3.6	96.2	95.6	3.8	4.4
Malabar spinach (n=8)	1.8	96.7	95.4	3.4	4.6
	3.6	96.9	95.4	3.1	4.6
EC		NS ⁱⁱ	NS	NS	NS
Species		***	***	***	***
EC $\times$ Species		NS	NS	NS	NS

ⁱ n= total number of samples used per treatment of the reported average

3.3.4 Shoot Mineral Nutrient Concentration with Different EC levels

Pooled data across species were analyzed to evaluate the main effect of EC on shoot N, P, K and nitrate concentrations (Table 3.6). A significant effect of EC was observed for shoot K and nitrate ($P < 0.01$), where shoot K and nitrate at elevated EC increased by 14% and 35%, respectively, compared to standard EC.

Table 3.7 presents shoot tissue N, P, K and nitrate concentrations among the species. A significant variation was observed among species for all four parameters. The highest shoot N concentration was observed in kale, which was comparable with lettuce; however, it was higher than basil and Malabar spinach by 14% and 27%, respectively. The highest shoot P was observed in basil, which was comparable with Malabar spinach, while it was higher than kale by 75% and lettuce by 55%. Additionally, lettuce displayed the highest shoot K concentration, which was comparable with Malabar spinach, but higher than kale by 91% and basil by 38%. The highest nitrate concentration was observed in kale, which was higher than basil by 123%, lettuce by 143%, and Malabar spinach by 105%.

Table 3.8 presents the effect of EC, species and their interaction on shoot N, P, K and nitrate concentrations. A significant interaction effect was observed for shoot nitrate concentrations, particularly in Malabar spinach, where nitrate concentrations increased by 249% at elevated EC compared to standard EC. In contrast, no significant interaction effect was observed for shoot N, P and K concentrations ($P > 0.05$). However, the shoot K concentration in basil increased by 32% at elevated EC compared to standard EC.

Table 3.6. Average nitrogen (N), phosphorus (P), potassium (K), and nitrate content across species with standard ($1.8 \text{ mS} \cdot \text{cm}^{-1}$) and elevated ($3.6 \text{ mS} \cdot \text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

EC ($\text{mS} \cdot \text{cm}^{-1}$)	N (% of DW) ⁱ	P% (% of DW)	K (% of DW)	Nitrate ($\text{mg} \cdot \text{kg}^{-1}$ FW)
1.8	5.1	1.1	7.4b ⁱⁱ	3401b
3.6	5.2	1.0	8.4a	4600a

P-value	NS ⁱⁱⁱ	NS	**	**
ⁱ N, P and K content was expressed in % based on dry weight basis				
ⁱⁱ Means within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)				
ⁱⁱⁱ NS, *, **, *** mean significant or significant at $p \leq 0.05, 0.01$ or 0.001 , respectively				

Table 3.7. Average tissue nitrogen (N), phosphorus (P), potassium (K), and nitrate content among basil 'Large Leaf', kale 'Winterbor F1', butterhead lettuce 'Salanova Red Butter', and Malabar spinach 'Rubra' with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

Species	N (% of DW) ⁱ	P (% of DW)	K (% of DW)	Nitrate ($\text{mg}\cdot\text{kg}^{-1}$ FW)
Basil	5.0b	1.4a	7.3b ⁱⁱ	3052b
Kale	5.7a	0.8c	5.3c	6821a
Lettuce	5.4ab	0.9bc	10.1a	2802b
Malabar spinach	4.5c	1.2ab	9.0a	3326b
P-value	*** ⁱⁱⁱ	**	***	***

ⁱ N, P and K content was expressed in % based on dry weight basis				
ⁱⁱ Means within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference test ($\alpha=0.05$)				
ⁱⁱⁱ NS, *, **, *** mean significant or significant at $p \leq 0.05, 0.01$ or 0.001 , respectively				

Table 3.8. Average tissue nitrogen (N), phosphorus (P), potassium (K), and nitrate content across basil 'Large Leaf', kale 'Winterbor F1', butter head lettuce 'Salanova Red Butter' and Malabar spinach 'Rubra' with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

Species	EC ($\text{mS}\cdot\text{cm}^{-1}$)	N (% of DW) ⁱ	P% (% of DW)	K (% of DW)	Nitrate ($\text{mg}\cdot\text{kg}^{-1}$ FW)
Basil (n=4) ⁱⁱ	1.8	5.1	1.6	6.3b ⁱⁱⁱ	3033a
	3.6	5.0	1.2	8.3a	3071a
Kale (n=4)	1.8	5.4	0.8	5.0a	6523a
	3.6	6.0	0.8	5.6a	7120a
Lettuce (n=4)	1.8	5.4	0.9	8.8a	2564a
	3.6	5.5	0.9	9.3a	3040a
Malabar spinach (n=3~4)	1.8	4.5	1.1	9.6a	1482b
	3.6	4.5	1.3	10.6a	5171a
EC		NS ^{iv}	NS	**	*
Species		***	**	***	***
EC $\times$ Species		NS	NS	NS	*
Sufficiency range ^v (Bryson & Mills, 2014; Mattson & Merrill, 2015)					Safe range ^{vi} (EU CoRegulation, 2011)
Basil		4.00-6.00	0.62-1.00	1.55-2.05	2500-5000
Kale		2.96-5.14	0.31-0.68	2.55-4.04	500-1000
Lettuce		4.50-6.50	0.30-0.80	6.0-10.0	1000-2500

ⁱ N, P and K content was expressed in % based on dry weight basis				
ⁱⁱ n= total number of samples used per treatment of the reported average				

ⁱⁱⁱMeans within a column followed by the same letter are not significantly different based on Tukey's honestly significant difference

test ($\alpha = 0.05$)

^{iv}NS, *, **, *** mean significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

^vLeaf tissue sufficiency ranges for N, P, and K are based on values for each species; comparison with whole shoot tissue (leaves and stems) should be interpreted with caution.

^{vi}Safe range

3.3.5 Color and Texture with Different EC levels

Pooled data across all species were analyzed to evaluate the main effect of EC on foliage color and texture (Table 3.9). No significant difference between EC levels was observed for color indices (L^* , a^* , and h°) and texture ($P < 0.05$).

Table 3.10 presents the color indices (L^* , a^* , and h°) and texture among the species. A significant difference was observed among the species for color indices (L^* , a^* , and h°) ($P < 0.001$) and texture ($P < 0.01$). The highest color L^* (lightness) was observed in Malabar spinach, which was 21%, 38%, and 41% higher than basil, kale, and lettuce, respectively. The highest color a^* (green to red axis) was observed in kale and which was 22%, 34%, and 46% higher than lettuce, basil, and Malabar spinach, respectively. Additionally, the highest color h° (hue angle) was observed in kale and which was 7%, 8%, and 17% greater than basil, lettuce, and Malabar spinach, respectively. For the texture, the highest value was observed in basil and which was 42% higher than kale.

Table 3.11 presents the effect of EC, species, and their interactions on color indices (L^* , a^* , and h°) and texture. No significant interaction effect was observed for any color indices (L^* , a^* , and h°) and texture ($P > 0.05$).

Table 3.9. Average color indices (L^* , a^* , and h°) and texture across species with standard ($1.8 \text{ mS} \cdot \text{cm}^{-1}$) and elevated ($3.6 \text{ mS} \cdot \text{cm}^{-1}$) EC levels in indoor growth tent throughout the study period

EC ($\text{mS} \cdot \text{cm}^{-1}$)	L^*	h°	a^*	Texture ($\text{N} \cdot \text{g}^{-1}$)
1.8	48.7	126.6	-15.8	14.9
3.6	47.8	127.4	-15.8	15.2

P-value	NS	NS	NS	NS
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NS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 3.10. Average color indices (L^* , a^* , and h°) and texture among basil ‘Large Leaf, kale ‘Winterbor F1’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

Species	L^*	h°	a^*	Texture ($\text{N}\cdot\text{g}^{-1}$)
Basil	49.0b ⁱⁱ	126.8b	-17.2c	17.5a
Kale	42.9c	135.2a	-11.2a	12.3b
Lettuce	42.0c	125.1c	-14.4b	15.4a
Malabar spinach	59.3a	116.1d	-20.6d	- ⁱ
P-value	*** ⁱⁱⁱ	***	***	**

ⁱNon-reported for the Malabar spinach

ⁱⁱMeans within a column followed by the same letter are not significantly different based on Tukey’s honestly significant difference test ($\alpha=0.05$)

ⁱⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 3.11. Average color indices (L^* , a^* , and hue) and texture across basil ‘Large Leaf, kale ‘Winterbor F1’, butterhead lettuce ‘Salanova Red Butter’ and Malabar spinach ‘Rubra’ across species with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels in indoor growth tent throughout the study period

Species	EC ($\text{mS}\cdot\text{cm}^{-1}$)	L^*	h°	a^*	Texture ($\text{N}\cdot\text{g}^{-1}$)
Basil	1.8	48.5	126.8	-17.2	16.5
	3.6	49.5	126.9	-17.2	18.5
Kale	1.8	43.4	135.6	-11.0	11.9
	3.6	42.3	134.8	-11.3	12.7
Lettuce	1.8	41.2	124.4	-14.7	16.3
	3.6	42.9	125.8	-14.0	14.5
Malabar spinach	1.8	61.9	114.8	-20.2	- ⁱ
	3.6	56.7	117.4	-20.9	-
EC		NS ⁱⁱ	NS	NS	NS
Species		***	***	***	**
EC $\times$ Species		NS	NS	NS	NS

ⁱNon-reported for Malabar spinach

ⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

3.3.6 Vitamin C, Anthocyanin, Total Phenolics, and Antioxidant Capacity with Different EC levels

Pooled data across all species were analyzed to evaluate the main effect of EC on vitamin C, anthocyanin, total phenolics, and antioxidant capacity (Table 3.12). No significant difference

was observed for vitamin C, anthocyanin, total phenolics, and antioxidant capacity between EC levels ($P < 0.05$).

Table 3.13 presents the vitamin C, total phenolics, and antioxidant capacity among the species. A significant difference was observed among the species for vitamin C ($P < 0.001$) and total phenolics ($P < 0.05$). The highest vitamin C was observed in kale, which was comparable with Malabar spinach, but was higher than basil by 99% and lettuce by 285%. Similarly, the highest total phenolics were observed in kale, which was comparable with Malabar spinach, but was higher than both basil and lettuce by 43%. However, antioxidant capacity was not varied among the species.

Table 3.14 presents the effect of EC, species, and their interactions on vitamin C, anthocyanin, total phenolics, and antioxidant capacity. No significant interaction effect was observed for vitamin C, total phenolics and antioxidant capacity between EC levels ($P > 0.05$).

Table 3.12. Average anthocyanin, vitamin C, antioxidant capacity, and total phenolics across species with standard ($1.8 \text{ mS} \cdot \text{cm}^{-1}$) and elevated ($3.6 \text{ mS} \cdot \text{cm}^{-1}$) EC levels in indoor growth tent throughout the study period

EC ($\text{mS} \cdot \text{cm}^{-1}$)	Anthocyanin ⁱ ($\text{mg} \cdot 100 \text{ g}^{-1} \text{ DW}$)	Vitamin C ($\text{mg} \cdot 100 \text{ g}^{-1} \text{ FW}$)	Antioxidant Capacity ($\mu\text{mol TE} \cdot 100 \text{ g}^{-1} \text{ DW}$)	Total Phenolics ($\text{mg GAE} \cdot 100 \text{ g}^{-1} \text{ DW}$)
1.8	166.6	46.5	3.4	0.8
3.6	89.0	47.9	3.2	0.8
P-value	NS ⁱⁱ	NS	NS	NS

ⁱAnthocyanin concentrations were determined for lettuce only

ⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 3.13. Average Vitamin C, antioxidant capacity, and total phenolics among basil ‘Large Leaf’, kale ‘WinterborF1’, butterhead lettuce ‘Salanova Red Butter’, and Malabar spinach ‘Rubra’ with standard ($1.8 \text{ mS} \cdot \text{cm}^{-1}$) and elevated ($3.6 \text{ mS} \cdot \text{cm}^{-1}$) EC levels in an indoor growth tent throughout the study period

Species	Vitamin C ($\text{mg} \cdot 100 \text{ g}^{-1} \text{ FW}$)	Antioxidant capacity ($\mu\text{mol TE} \cdot 100 \text{ g}^{-1} \text{ DW}$)	Total Phenolics ($\text{mg GAE} \cdot 100 \text{ g}^{-1} \text{ DW}$)
Basil	36.8b ⁱ	4.0	0.7b
Kale	73.5a	2.9	0.8ab
Lettuce	19.1c	3.1	0.7b
Malabar spinach	59.5a	3.2	1.0a
P-value	*** ⁱⁱ	NS	*

ⁱMeans within a column followed by the same letter are not significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$)

ⁱⁱNS, *, **, *** mean non-significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

Table 3.14. Average Vitamin C, antioxidant capacity, and total phenolics across basil ‘Large Leaf’, kale ‘WinterborF1’, butter head lettuce ‘Salanova Red Butter’, and Malabar spinach ‘Rubra’ with standard (1.8 mS·cm⁻¹) and elevated (3.6 mS·cm⁻¹) EC levels in an indoor growth tent throughout the study period

Species	EC (mS·cm ⁻¹)	Vitamin C (mg·100 g ⁻¹ FW)	Antioxidant capacity (μmol TE ·100 g ⁻¹ DW)	Total Phenolics (mg GAE ·100 g ⁻¹ DW)
Basil	1.8	35.5	3.9	0.7
	3.6	38.1	4.0	0.7
Kale	1.8	72.9	2.9	0.9
	3.6	74.0	2.8	0.8
Lettuce	1.8	18.3	3.4	0.7
	3.6	20.0	2.8	0.6
Malabar spinach	1.8	59.4	3.3	0.9
	3.6	59.5	3.1	1.1
EC		NS	NS	NS
Species		***	NS	*
EC × Species		NS	NS	NS

NS, *, **, *** mean significant or significant at $p \leq 0.05$, 0.01 or 0.001, respectively

3.4 Discussion

3.4.1 Plant Height, Leaf Number and SPAD in Response to EC levels

EC levels in the current study influenced plant height and SPAD value. The highest plant height at elevated EC across species in the current study contrasts with the findings of Conversa et al. (2021), who reported reduced plant height in lettuce at EC 3.5 dS·m⁻¹ compared to 2.5 dS·m⁻¹. This discrepancy might be due to differences in hydroponic systems. Conversa et al. used floating and ebb-and-flow systems, which can expose roots to greater osmotic stress due to intermittent wettings and full submersion. In contrast, the NFT system used in the current study had a continuous thin film of NS, providing consistent root aeration, more stable root zone conditions, and reduced salt stress. These findings are supported by Al-Kinani et al. (2021) who reported greater plant height in five lettuce varieties grown in NFT compared to those in an ebb-and-flow system. Additionally, elevated EC enhanced SPAD across species in the current study. Higher SPAD with elevated EC aligns with the findings of Kappel et al. (2021), who reported

that higher SPAD at high EC of $10 \text{ dS}\cdot\text{m}^{-1}$ compared to low EC $<1.3 \text{ dS}\cdot\text{m}^{-1}$. Among the tested species, Malabar spinach displayed higher SPAD at elevated EC during the whole study. Slight yellowing of old and younger leaves in Malabar spinach at standard EC suggests the presence of interveinal chlorosis (Figure 3.3), which might be due to an inadequate amount of N, Mg, Fe, and Mo (Croze, 2019). These findings indicate that standard EC may not sufficiently meet the nutrient demands of Malabar spinach. Further research is needed to evaluate the response of Malabar spinach to EC levels beyond elevated EC, as the plant demonstrated a positive growth trend under higher EC. Additionally, studies with extended growth periods are recommended, considering that the typical harvest time for Malabar spinach is 70 days (Qiu & Liu, 2020). Substrate-based hydroponic system, such as Dutch bucket can be explored as they can support crops with a longer period of growth cycle, such as tomato and cucumber (Abul-Soud et al., 2021).

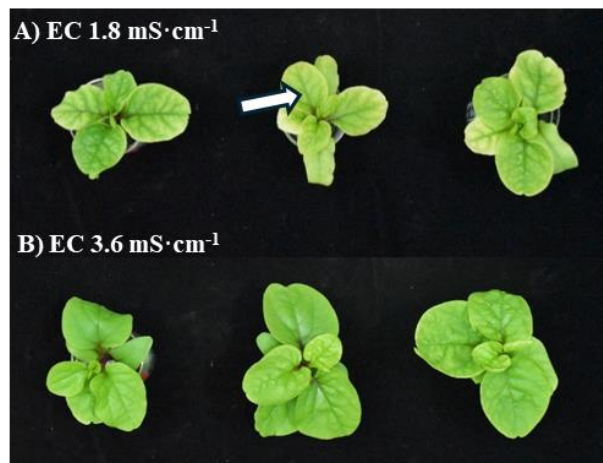


Figure 3.3. Visual Comparison of Malabar spinach grown with standard ($1.8 \text{ mS}\cdot\text{cm}^{-1}$) and elevated ($3.6 \text{ mS}\cdot\text{cm}^{-1}$) EC levels

However, in the current study, plant height and leaf number in basil and kale remained stable, aligning with the findings of Walters & Currey (2018), who reported that increasing EC from 0 to $4 \text{ dS}\cdot\text{m}^{-1}$ did not show any variation in terms of height in several basil species.

3.4.2 Biomass accumulation in Response to EC levels

In the current study, shoot fresh weight was influenced by EC levels across all species. A significant interaction between EC and species for both shoot and root dry weight indicates that species responded differently to EC levels. In kale, higher shoot and root dry weight at elevated EC aligns with the results of Yang et al. (2024), who reported that higher shoot fresh weight and shoot dry weight at EC 2.1 $\text{mS}\cdot\text{m}^{-1}$ compared to 1.2 $\text{mS}\cdot\text{m}^{-1}$. This may be attributed to enhanced photosynthetic activity under higher EC levels in kale (Yang et al., 2024). However, the shoot and root dry weight of the remaining species (basil, lettuce, and Malabar spinach) was not affected by EC levels. This observation is consistent with previous studies by Currey et al. (2019) and Walters & Currey (2018), who reported that EC levels (0.5, 1, 2, 3, and 4 $\text{dS}\cdot\text{m}^{-1}$) did not show variation for fresh and dry mass in basil, dill, parsley, and cilantro. Similarly, Samarakoon et al. (2020) observed that fresh weight in lettuce remained consistent between EC 1.8 and 2.0 $\text{mS}\cdot\text{m}^{-1}$. Hosseini et al. (2021) reported that shoot and root biomass in lettuce and basil significantly increased up to 0.9 and 1.2 $\text{dS}\cdot\text{m}^{-1}$, but did not increase further at EC 2.1 $\text{dS}\cdot\text{m}^{-1}$.

Collectively, these findings highlight species-specific responses to EC, with kale showing improved biomass at elevated EC while basil, lettuce, and Malabar spinach showed no additional biomass gain at this level compared to standard EC. This suggests that the optimal EC for these latter species may fall between standard and elevated EC, warrant further research using intermediate EC levels to determine species-specific nutrient requirements for maximizing growth and resource efficiency. In contrast, kale may benefit from exploring EC levels even higher than elevated EC to further enhance biomass production.

3.4.3 Tissue Mineral Nutrient Accumulation in Response to EC levels

EC levels did not affect shoot N and P concentration but did influence shoot K concentration in the current study. This finding aligns with the results of Currey et al. (2019), who studied EC (0.5, 1, 2, 3, and 4 dS·m⁻¹) and DLI (7 and 18 mol·m⁻²·d⁻¹) on nutrient accumulation in cilantro, dill, and parsley. They reported that increasing EC alone had no significant effect on shoot N and P content in any of the species. However, in dill, a significant interaction between DLI and EC was observed for shoot K concentration. Under a DLI of 7 mol·m⁻²·d⁻¹, shoot K increased by 4.2% at EC 0.5 dS·m⁻¹ and by 5.8% to 6.2% as EC increased from 1 to 4 dS·m⁻¹.

In our study, elevated EC increased shoot K concentrations by 14% to standard EC. This aligns with the findings of Voutsinos-Frantzis et al. (2022), who reported that shoot K content in hydroponically grown spiny chicory (*Cichorium spinosum* L.) in a floating raft system increased by 10% when EC increased from 2.4 to 3.6 dS·m⁻¹, while N and P remained unaffected. Interestingly, in basil, the increase of 24% shoot K in elevated EC than standard EC in the current study contrasts with the findings of Walters & Currey (2018), who reported a decrease in shoot K concentration in sweet basil as EC increased from 1 to 4 dS·m⁻¹.

This discrepancy may be due to differences in cultivar morphology and nutrient uptake behavior. Walters and Currey (2018) used basil ‘Sweet’, while the current study used ‘Large Leaf’, which is known to have broader leaves and short internodes. These traits help prevent lodging in NFT hydroponic systems (Walters & Currey, 2015). The larger leaf area of basil ‘Large leaf’ may enhance nutrient uptake efficiency, possibly explaining the increased shoot K concentration observed at elevated EC in our study. However, despite the increase in shoot K, biomass production in basil ‘Large leaf’ did not improve under the elevated EC treatment.

In addition to cultivar differences, variations in growing environments may also contribute to the contrasting results. Our study was conducted in an indoor growth tent, whereas Walters & Currey (2018) used a greenhouse setting. As noted by Solis-Toapanta et al. (2020), environmental factors such as humidity, airflow, light intensity can significantly influence plant growth and nutrient uptake in hydroponic system. Supporting this, Singh et al. (2019) reported that basil ‘Large leaf’ had higher shoot dry weight with fertilizer (5N–4.8P–21.6K) at an EC range of 1.5 to 2.5 dS·m⁻¹ compared to other basil cultivar such as ‘Lemon’ and ‘Sweet’.

Shoot tissue (including leaves and stems) was analyzed for N, P, and K content and compared with the leaf tissue sufficiency ranges reported by Bryson & Mills (2014). In basil, N concentrations at both EC levels (standard and elevated) were within the sufficient range, while P at both EC levels exceeded the upper limit by 19-57%. In kale, shoot N, P, and K content at both EC levels were above the sufficient range. Lettuce shoots contained adequate N and K under both EC levels, but P was 15% and 10% above the recommended range at standard EC and elevated EC, respectively. For Malabar spinach, published sufficiency ranges are not yet available, underscoring the need for additional research to determine optimal EC levels for this crop in a hydroponic system.

3.4.4 Health Implications of Lower K and Nitrate

Chronic kidney disease (CKD) has been a global health concern, as 1.2 million people died from CKD in 2017 (Bikbov et al., 2020). It has been difficult for CKD patients to excrete 90% of K intake by the kidney to maintain serum K concentration within the normal range (Choi & Ha, 2013; Putcha & Allon, 2007). In such conditions, nutritional approaches can play a crucial role in improving the physiological conditions of these patients by using low-K containing vegetables and fruits (D’Imperio et al., 2019). The lower K content in basil and kale in the

current study supports that these leafy green species may be suitable for inclusion in the low-K diet for patients with kidney-related diseases. Additionally, the lower K content in the basil, kale, lettuce, and Malabar spinach at standard EC highlights that EC $1.8 \text{ mS}\cdot\text{m}^{-1}$ could be a potential strategy to produce low K-containing leafy greens in the hydroponic production within indoor growth environments.

Furthermore, nitrate concerns in leafy greens are increasing under elevated EC. Because high amounts of nitrate can result in life-threatening diseases such as methemoglobinemia or gastric cancer, it is better to keep nitrate under a certain threshold (Bryan et al., 2012; Chan, 2011). The Scientific Committee on Food of European Commission has set a guideline for the acceptable daily intake (ADI) of nitrate of 0 to $0.35 \text{ mg}\cdot\text{kg}^{-1}$ of body weight and has set an ADI of nitrite of 0 to $0.07 \text{ mg}\cdot\text{kg}^{-1}$ of body weight (Santamaria & Elia, 1997). Nitrate accumulation is particularly influenced by fertilizer management, environmental factors (temperature, light, CO_2), cultivar, and species (Colla et al., 2018).

In our study, shoot nitrate concentrations rose at elevated EC, a trend also observed by Ding et al. (2018), Fallovo et al. (2009), Voutsinos-Frantzis et al. (2022), and Yang et al. (2021), who reported that nitrate concentration increased with increasing NS concentration. Notably, total N did not increase in parallel with nitrate at elevated EC, suggesting the limited nitrate assimilation capacity under such EC levels. When assimilation is restricted, plants sequester excess nitrate in vacuoles rather than converting it into organic N compounds (amino acids and protein). This finding aligns with the results of Voutsinos-Frantzis et al. (2022), where EC 2.4 and $3.6 \text{ dS}\cdot\text{m}^{-1}$ influenced nitrate without affecting the total N in hydroponically grown chicory using a floating raft system. Scheible et al. (1997) noted that during limited nitrate assimilation, vacuolar nitrate is retained and balanced by organic acids to prevent cellular alkalization.

Plant architecture and lighting conditions may have contributed to this outcome. In the current experiment, a vertical NFT system with four stacked horizontal channels was used. Because illumination came only from above, light intensity likely declined from the upper to the lower canopy, reducing photosynthesis in shaded leaves (Kozai, 2013). As nitrate reduction is dependent on light to convert nitrate into amino acids (Cavauiolo & Ferrante, 2014), diminished light in lower shaded leaves could have slowed nitrate reduction and led to higher nitrate accumulation in shoot tissues. Further research is required on light distribution within stacked hydroponic systems to identify practices that minimize nitrate build-up while maintaining plant growth.

Tissue nitrate content in the current study was paired with the safety thresholds established by European Commission (EU) Regulations No 1258/2011 (Colla et al., 2018). Nitrate concentrations in basil at both EC levels (standard and elevated EC) and lettuce at standard EC were within the safe range. The lower nitrate concentration in lettuce at standard EC was further supported by Sago & Shigemura (2018), who reported that nitrate concentration at EC 1.2 and 2 dS·m⁻¹ remained within the safe range. Interestingly, their study also observed a safe nitrate range at EC 3.0 dS·m⁻¹ (Sago & Shigemura, 2018). This discrepancy is likely due to cultivar difference, as they utilized ‘Pansoma’, while our study used ‘Salanova Red Butter’ (Sago & Shigemura, 2018). In addition, Sago & Shigemura (2018) used a deep flow technique (DFT) hydroponic system with floating panels, whereas our study used an NFT. Differences in the hydroponic system could also explain variations in nitrate accumulation, as DFT maintains the greater oxygenation and buffering capacity that might influence the nitrate uptake compared to the continuous flow of thin film of water in NFT system (Lu & Shimamura, 2018).

No nitrate safe range was available for Malabar spinach; however, when considering the safe range limit for lettuce as a reference, nitrate level in Malabar spinach remained within safe range at standard EC, but not at elevated EC. In contrast, kale at both EC levels was out of the safe range. This suggests that further research is needed on kale and Malabar spinach to identify safe nitrate concentrations.

3.4.5 Color and Texture

It is important to consider factors such as color, size, texture, and taste, which play a role in the successful marketing of crop species (Mampholo et al., 2016; Renna et al., 2017). EC in the current study did not influence color indices (L^* , a^* , and h°), which aligns with the findings of Conversa et al. (2021), who reported that EC levels (2.5 and 3.5 $\text{dS}\cdot\text{m}^{-1}$) did not affect L^* and h° in hydroponically grown romaine lettuce and endive (*Cichorium endivia*).

The texture or firmness in the current study was not affected by EC levels, indicating that EC did not influence the softness or firmness of the leaves. These findings contrast with a previous study by Cuppett et al. (1999), who reported that lettuce ‘Grand Rapids’ became firmer with increasing nitrogen concentration in NS. Conversely, leaves of Pak choi became softer with an increase in EC from 2 to 5 $\text{dS}\cdot\text{m}^{-1}$ compared to EC 1 and 2 $\text{dS}\cdot\text{m}^{-1}$ (Oliveira et al., 2024). The discrepancy in texture might be due to different growing conditions (greenhouse in their study while indoor growth tent in our study) and different hydroponic systems (NFT and substrate-based in their study, while we used NFT). In addition, Amoruso et al. (2022), reported that leaf texture in sea fennel (*Crithmum maritimum L.*) decreased with increasing salinity (150 mM NaCl) compared to low salinity (9 mM NaCl) in a floating hydroponic system. The discrepancy in our study compared to their study might be due to different mechanisms behind EC adjustment and different growing conditions, and the hydroponic system. High EC came from

salt stress in their study using floating beds in a greenhouse, while EC in the current study was adjusted using balanced nutrient concentrations in an NFT hydroponic system in an indoor growth tent. This suggests that leaf texture responses to EC levels are species-specific and influenced by the source of EC, growing facilities, and the hydroponic system used.

3.4.6 Phytochemical Concentrations

Vitamin C is one of the major nutritional quality factors, and more than 90% of vitamin C in the human diet is obtained through vegetables and fruits (Lee & Kader, 2000). Vitamin C has a role in the biosynthesis of collagen, carnitine, and catecholamines (Frei & Traber, 2001).

The vitamin C in the current study across all species or within an individual species was not affected by EC levels, suggesting that standard EC did not reduce vitamin C concentrations. However, a previous study by Quy et al. (2018) reported that the highest vitamin C was observed at EC $1.5 \text{ mS} \cdot \text{cm}^{-1}$ compared to 0.5, 1.0, 2.0, and $2.5 \text{ mS} \cdot \text{cm}^{-1}$ in lettuce. This discrepancy might be due to cultivar differences and different growing conditions such as they employed ‘Rapid Red Lettuce’ using a vertical hydroponic system in a greenhouse, while we utilized ‘Salanova Red Butter’ using a vertical hydroponic system in an indoor growth tent. Since higher temperature (Walters & Currey, 2019), higher light availability (Beaman et al., 2009) and high nutrient uptake in the greenhouse compared to indoor, which could explain the differentiation in vitamin C concentration. Similarly, Mampholo et al (2016) reported that higher vitamin C at EC $2.0\text{-}2.4 \text{ mS} \cdot \text{cm}^{-1}$ in red oak lettuce varieties ‘Multitired 4’ and ‘Cantariz’. This suggests that the vitamin C response to EC levels in lettuce is cultivar-specific. In kale in the current study at both EC levels (standard and elevated) did not affect the vitamin C concentration, which contrasts with the findings of Yang et al. (2024), where they reported that vitamin C decreased as EC increased from EC 1.5 to $2.1 \text{ dS} \cdot \text{m}^{-1}$. The differences might be due to growing conditions, DLI

difference and cultivar differences, as they employed ‘Red Russian’ in the greenhouse using $28.7 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, while we used ‘Winterbor F₁’ in an indoor growth tent using $14.4 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$.

Rouphael et al. (2018) reported that phytochemicals can be influenced by cultivars and light conditions.

EC effect on the anthocyanin content in red butter lettuce remained stable, which aligns with the findings of Yang et al. (2021), who reported that anthocyanin in the arugula ‘Standard’ decreased between EC $1.2 \text{ dS}\cdot\text{m}^{-1}$ and higher EC (1.5 , 1.8 , and $2.1 \text{ dS}\cdot\text{m}^{-1}$). This suggests that the EC effect on anthocyanins is limited.

Total phenolic content and antioxidant capacity in the current study was not influenced by EC levels, which aligns with the findings of Hamilton & Fonseca (2010), who reported that no consistent trend of total phenolics in response to EC levels (1.5 , 3.5 , 5.5 , 7.5 and $9.5 \text{ dS}\cdot\text{m}^{-1}$) in different cultivars of arugula such as ‘Sylvetta’ and ‘Astro’, and cultivars of lettuce ‘Presto’.

This highlights that cultivar, species, and environment together determine how EC levels influence phytochemical quality traits.

3.5 Conclusion

In conclusion, EC levels influenced plant height and SPAD value in leafy greens with species-response. Plant height increased with the elevated EC of $3.6 \text{ mS}\cdot\text{cm}^{-1}$. SPAD values were increased by elevated EC of $3.6 \text{ mS}\cdot\text{cm}^{-1}$, particularly in Malabar spinach. Shoot fresh weights were increased by elevated EC across all species. Shoot and root dry weights were increased by elevated EC, particularly in kale. Shoot tissue analysis revealed high K concentration at elevated EC of $3.6 \text{ mS}\cdot\text{cm}^{-1}$. Lower K content in basil, kale, lettuce, and Malabar spinach at a standard EC of $1.8 \text{ mS}\cdot\text{cm}^{-1}$ would be the potential strategy to produce lower K-containing vegetables for the CKD patients. Standard EC of $1.8 \text{ mS}\cdot\text{cm}^{-1}$ reduced the nitrate levels in basil, lettuce and Malabar

spinach that fall within the safe range limits, while kale consistently surpassed recommended safe nitrate levels regardless of treatment. Postharvest quality, including the color, texture and nutritional quality (vitamin C, anthocyanin, total phenolics, and antioxidant capacity) was not affected by EC levels. Although elevated EC of $3.6 \text{ mS}\cdot\text{cm}^{-1}$ increased shoot and root dry weight in kale, it also accumulated the highest nitrate levels beyond the safety threshold limit, which indicates a food safety concern. Slight yellowing of old and young leaves in Malabar spinach at standard EC of $1.8 \text{ mS}\cdot\text{cm}^{-1}$ and similar shoot fresh weight under both EC levels (standard and elevated) suggests further research is needed to optimize EC management for this emerging crop species. Similarly, high wire production systems should be explored for Malabar spinach to support its vining growth habit, extend harvest duration to increase yield, and enhance the optimal foliage color under higher EC levels. Overall, these findings highlight the importance of crop-specific EC management to optimize yield, nitrate safety, and nutritional quality, supporting sustainable hydroponic production of diverse leafy green species.

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