HortScience 60(10):1744–1751. 2025. https://doi.org/10.21273/HORTSCI18774-25

Boosting Hydroponic Production of Kale and Arugula by Managing Dissolved Oxygen

Kuan Qin and Rhuanito Soranz Ferrarezi
Department of Horticulture, University of Georgia, 1111 Miller Plant Sciences Building, Athens, GA 30602, USA

Keywords. deep water culture, energy cost–economic analysis, gas exchange, root–shoot ratio, sensor-based control system

Abstract. **The dissolved oxygen (DO) level in the hydroponic solution is an important factor influencing plant development and water and nutrient uptake. There is limited information on the optimal DO concentrations and methods to precisely control the levels for leafy greens growth in hydroponics. In this greenhouse study, we built a real-time adjusted DO enrichment system using different techniques (oxygen generator and infuser) and sensor-based monitoring system in deep water culture hydroponics. We evaluated the effects of three DO enrichment levels (10, 15, and 20 mg·L^{-1}) on arugula (*Eruca vesicaria* 'Astro' and 'Esmee') and kale (*Brassica oleracea* 'KX-1' and 'Red Russian') growth in comparison with the traditional air pump–based aeration system. Weekly measurements were taken for plant height, leaf greenness, fresh and dry biomass, root–shoot ratio, until final harvest. We also measured leaf gas exchange, soluble solids content (SSC), and nutrient concentration at the end. Additionally, energy costs and yield returns were evaluated. The results showed that DO enrichment level higher than 15 mg·L^{-1} benefited arugula biomass (63% to 191% improvement) but had no significant effect on kale growth. The treatments did not alter arugula or kale root-to-shoot ratios, SSC, or gas exchange, and the impact on plant nutrient content varied. DO enrichment treatments incurred over 140% higher energy costs (electricity) compared with the control (air pump only), with only arugula grown under DO levels of 20 mg·L^{-1} showing net economic returns ($0.14 increased per plant) that justified the increased energy expenditure. This study demonstrated using DO enrichment to benefit hydroponic plant production was crop- and concentration-specific.**

The dissolved oxygen (DO) level in hydroponics is crucial for the root zone, as it directly affects plant growth due to the oxygen requirements of root respiration. Variations in DO levels in irrigation water can significantly affect plant morpho-physiological responses: DO levels below 3 to 4 mg·L^{-1} often inhibit root and shoot growth due to root hypoxia and browning, decreased stomatal conductance, and decreased water consumption (Moreno Roblero et al. 2020); when DO levels are maintained above critical low levels, this facilitates plant growth and water and nutrient uptake, and reduces pathogen incidence (Hendrickson et al. 2022; Ragaveena et al. 2021).

Received for publication 5 Jun 2025. Accepted for publication 25 Jul 2025.
Published online 8 Sep 2025.
This research was funded by the University of Georgia Department of Horticulture, the University of Georgia College of Agricultural and Environmental Sciences, and the University of Georgia Office of the Senior Vice President for Academic Affairs and Provost. We thank Matthew Joseph Housley and Timothy (Lei) Dunn for technical support, Freeman Agnew and Jiffy (Lorain, OH, USA) for donating the plant plugs, and Dr. Cari Peters and J.R. Peters for the fertilizer donation.
R.S.F. is the corresponding author. E-mail: ferrarezi@uga.edu.
This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/).

Several studies have evaluated how elevated DO levels affect lettuce (*Lactuca sativa* L.) growth, In one study, raising the DO concentration from 6.5 to 8.5 mg·L^{-1} significantly boosted lettuce (cv. Grand Rapids TBR) growth—increasing plant height, leaf area index, chlorophyll content, net photosynthetic rate, dry matter accumulation, vitamin C content, soluble protein content, yield, and water use efficiency (Ouyang et al. 2020). Similarly, Kurashina et al. (2019) compared two oxygenation methods in hydroponic systems: while the common practice uses an air stone to maintain DO saturation at 9 mg·L^{-1} (at 20 °C), applying pure oxygen to create DO supersaturation (up to 36 mg·L^{-1}) further stimulated lettuce (cv. Green wave) leaf growth with higher leaf length, number, and biomass, but no differences were found on root length and leaf chlorophyll contents. Although several studies have shown that high DO levels enhance lettuce growth, other research suggests that there is a threshold beyond which additional DO does not further benefit shoot and root development. For example, lettuce (cv. Ostinata) grown under a supersaturated DO level (16.8 mg·L^{-1}) had no significant improvement compared with a saturated DO level (8.4 mg·L^{-1}) (Goto et al. 1996). Therefore, exploring the optimal DO level that maintains a balanced root and shoot growth is essential.

Several practices have been used to adjust DO levels in hydroponic solutions. The DO level is normally inversely correlated with water temperature—higher water temperature holds lower DO, while lower water temperature holds higher DO—and growers usually adjust the DO level by controlling solution temperatures. Al-Rawahy et al. (2019) used chiller cooling coils to lower the root zone temperature of hydroponic cucumber (*Cucumis sativus* L.) from 33 to 22 °C and found that DO level increased from 5 to 8 mg·L^{-1} and plant root showed 44% higher oxygen consumption with 23% to 69% higher plant height, leaf number and area, chlorophyll content, yield, and biomass. Although lowering the solution temperature offers multiple benefits, achieving enriched DO alone through this method requires a large amount of electricity. In addition to potentially controlling solution temperature, liquid oxygen fertilization using H_2O_2 has been shown to burst increase DO concentration in the solution and lead to a higher snap bean (*Phaseolus vulgaris*) N and P uptake rates (Liu et al. 2022). Additionally, applying O_3 using ozone generators to nutrient solutions has been considered a sanitization treatment to inactivate microorganisms due to the generation of reactive oxygen species, such as the OH^-, H_2O_2, and O_2^-. On the other hand, O_3 can also be used to supply oxygen in nutrient solution to saturate the DO level, and a proper level of supplemental O_3 has shown to improve tomato, pepper, and chard growth (Machuca et al. 2023). However, these oxygen generation methods have been tested in substrate-based systems for fruiting crops and are less reported in water-based systems for growing leafy greens. More recently, DO enrichment using oxygen infusion or nanobubble techniques (NBT) have been developed and tested: DO level could reach up to 30 mg·L^{-1} using NBT as compared with control (10 mg·L^{-1}) that used traditional air stone-based aeration (DeBoer et al. 2024).

Despite research on the effects of various DO concentrations on plant growth and methods to achieve DO enrichment, there are fewer assessments of whether DO enrichment benefits leafy greens other than lettuce. Additionally, there are limited comparisons to determine the optimal DO levels for leafy green growth in hydroponics, assessments of real-time adjustments to DO fluctuations, and economic evaluations of DO enrichment practices. The objectives of the study are to: (1) build a real-time adjusted DO enrichment system using oxygen generator and DO enrichment techniques in a deep water culture (DWC) system; (2) evaluate effects of three different DO enrichment levels (10, 15, and 20 mg·L^{-1}) on kale and arugula growth in comparison to the control; and (3) evaluate the energy cost and yield return for economic analysis.

Materials and Methods

Study location, environment, and hydroponic system. This study was conducted in a greenhouse at the University of Georgia (College of Agricultural and Environmental

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

Sciences, Department of Horticulture, Controlled Environment Agriculture Crop Physiology and Production Laboratory) in Griffin, GA, USA (33.26°N, 84.28°W) for two cycles. Environmental conditions inside the greenhouse were controlled by a Wadsworth control system (EnviroSTEP; Arvada, CO, USA) and monitored using a temperature and relative humidity sensor (HMP60; Vaisala, Vantaa, Finland) and a photosynthetic photon flux density light sensor (SQ-610 ePAR; Apogee Instruments, Logan, UT, USA) connected to a datalogger (CR1000X; Campbell Scientific, Logan, UT, USA). Average air temperature, vapor pressure deficit (VPD), and daily light integral (DLI) for the first cycle (Jul 2024) were 26.7 °C, 0.7 kPa, 24.4 mol·m^{-2}·d^{-1}, respectively; and for the second cycle (Aug 2024) were 27.1 °C, 1.1 kPa, 28.7 mol·m^{-2}·d^{-1}, respectively. The DWC hydroponics system was composed of twelve 190-L 122 × 122 × 20-cm (width × length × height) trays (Botanicare, Vancouver, WA, USA) covered with 122 × 122 × 2.5-cm (width × length × height) unfaced extruded polystyrene foam insulation boards (Owens Corning, Toledo, OH, USA) drilled with 6 × 6 holes with a 4.4-cm diameter.

Growth conditions and plant material. Two crops each with two cultivars (Johnny's Selected Seeds, ME, USA) were used in this study: arugula (*Eruca vesicaria* L., 'Astro' and 'Esmee' with different leaf shapes) and kale (*Brassica oleracea* L., 'KX-1' and 'Red Russian' with different color leaves). The seeds were seeded in plugs composed of 75% peat and 25% coir (Preforma; Jiffy, Lorain, OH, USA). The plugs were placed inside the greenhouse under overhead irrigation for 10 d, then were transplanted into the DWC trays using 4.4-cm-top diameter × 3.2-cm-bottom diameter × 4.8-cm-deep net cups (Orimerc Garden, Seattle, WA, USA), and allowed to grow for 3 weeks.

Treatments. Three DO enrichment levels (10, 15, and 20 mg·L^{-1}) and a control were used in this study, with a total of four treatments. DWC trays under control treatment were aerated by four 5.1 cm air stones (Aquaneat, Madison, WI, USA) connected by 0.8- × 0.5-cm (outside × inner diameter) clear extruded acrylic tubes (Dernord; Tangxia, Dongguan, China) to a 3.75 L/s at 0.048 MPa aeration pump (120 V and 3.0 A) with a 1.3 cm outlet (EcoAir 7; EcoPlus, Vancouver, WA, USA).

DO enrichment was achieved using a recirculating system (Supplemental Fig. 1) designed to maintain a high DO concentration. The system included a 1140-L stock tank connected to an oxygen generator (Pro-10; Oxidation Technologies, Inwood, IA, USA), an oxygen infusion tube (TOOB Craft; Bio-Therm, Cotati, CA, USA), and a sump pump with a capacity of 2.6 L/s (Acquaer, Louisville, KY, USA). The stock tank supplied an inline system composed of DWC trays with water input and drainage lines. For DO enrichment treatments, the water input line was equipped with a submersible pump (NK-1; Little Giant, OK City, OK, USA), a relay (Digital Loggers, Santa Clara, CA, USA), and oxygen sensors (OXYBase WR-RS232; PreSens, Germany) connected to a CR1000X datalogger. Every 5 min, if the DO level dropped below a set threshold (e.g., DO 10 mg·L^{-1}), the relay activated the pump for 1 min to send oxygen-enriched water into the targeted DWC trays. The drainage line, positioned at a fixed height, facilitated water exchange within the trays.

Fertilization and solution monitoring. The DWC trays and stock solution were filled with a 15N–2.2P–16.6K fertilizer solution (Jack's Nutrients 15-5-20 FeED; JR Peters, Allentown, PA, USA) containing macro- and micronutrients based on a 150 mg·L^{-1} N concentration. Solution pH and electrical conductivity (EC) were monitored every other day using pH and EC meters (HI98129; Hanna Instruments, Woonsocket, RI, USA) and kept at the ranges of 5.5 to 7.0 and 1.2 to 1.5 dS·m^{-1}, respectively.

Dissolved oxygen monitoring. Real-time DO concentrations from each treatment and the stock solution (n = 5) were monitored and recorded by oxygen sensors and the datalogger. A hand-held dissolved oxygen meter (HI98193; Hanna Instruments) was used three times per week to confirm the DO readings from the sensors. The total turned-on times of the water pump from each treatment were also recorded automatically by the datalogger.

Plant growth measurements. For each experiment cycle, weekly measurements from transplanting to the end of the study were conducted for plant height, leaf greenness [Soil Plant Analysis Development (SPAD) chlorophyll meter SPAD-502Plus; Konica Minolta, Tokyo, Japan] with three leaves averaged; then the plants were harvested, roots were washed, shoot fresh weight was determined by weighing the canopy biomass, shoot and root dry weight was determined after oven drying at 80 °C for 3 d, and root–shoot ratio was calculated by root dry weight/shoot dry weight. At the end of the study (3 weeks after transplanting), additional measurements were taken: before harvesting, leaf net assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) were measured using a portable photosynthesis system (LI-COR 6800; LI-COR Biosciences, Lincoln, NE, USA), instantaneous water use efficiency (WUE) was calculated by A/E, while intrinsic WUE was calculated by A/g_s; after harvesting, leaf soluble solids content (%) was measured using a pocket

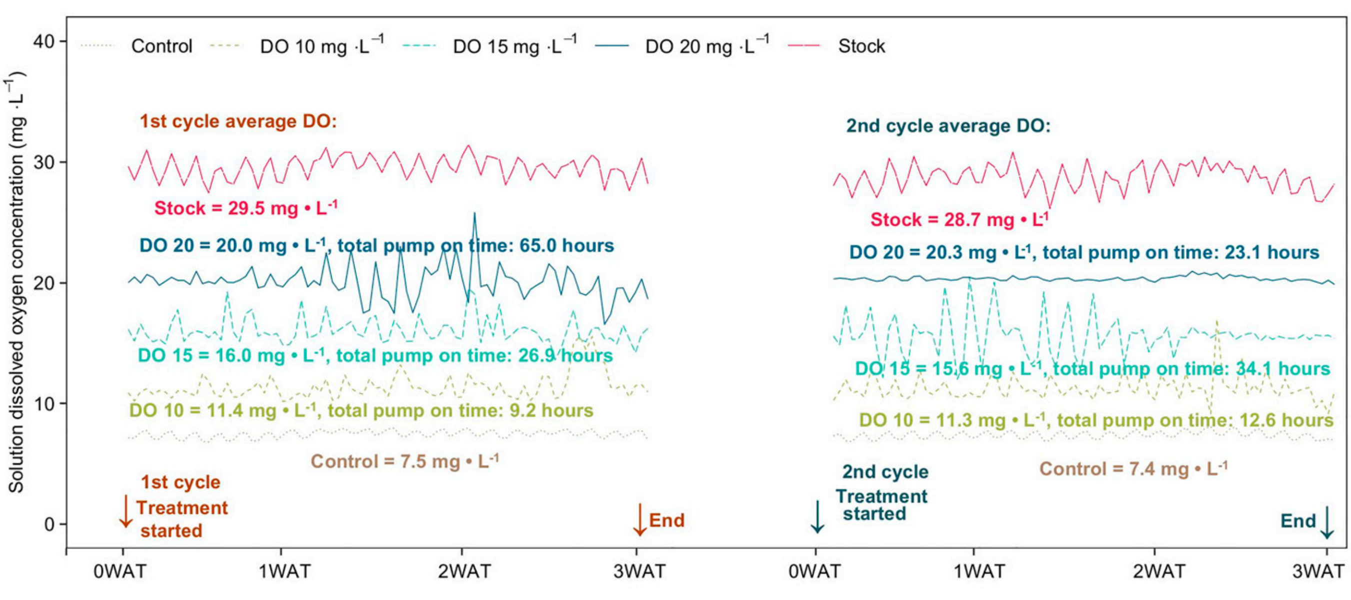

Fig. 1. Real-time monitoring of dissolved oxygen (DO) concentrations and total water pump turned-on time from treatments (control and DO 10, 15, and 20 mg·L^{-1}) and stock solutions during the study period and two cycles.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

brix-acidity meter (PAL-BX; Atago, Tokyo, Japan); after oven drying, leaf samples were collected and shipped to a commercial laboratory (Waters Agricultural Laboratories, Camilla, GA, USA) for tissue mineral concentration analysis of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, and zinc.

Treatment economic analysis. At the end of the study, total energy cost from each treatment was calculated considering the total turned-on time of all the devices and their electrical power: oxygen generator (700 W), sump pump for DO enrichment recirculation in the stock solution (172 W), water pump to DWC trays (100 W), and air pump for aeration (360 W). The percentage increase or decrease for energy cost and yield return compared with elevated DO treatments to control was calculated, and the economic values of energy cost, yield return, and net return (net return of yield value minus energy cost) were estimated based on price information from U.S. Energy Information Administration (average retail price of electricity was \$0.134/kWh) (US Energy Information Administration 2025), TRIDGE (average arugula retail price was \$11.02/kg) (TRIDGE 2025), and USDA National Retail Report–Specialty Crops (average kale retail price was \$2.23/kg) (US Department of Agriculture 2025) in Jul and Aug 2024.

Experimental design and statistical analysis. For each crop and cultivar (arugula Astro and Esmee, kale KX-1 and Red Russian), the study was arranged on a completely randomized design with four treatments (control, DO10, DO15, and DO20) and two consecutive cycles considered as treatment factors. For each cycle, each treatment was randomly assigned to three DWC trays (as three replications, $n = 3$) with a total of 12 trays. Each cultivar was randomly planted into the trays with a total of nine plants per cultivar per tray. Analysis of variance (ANOVA) regarding the fixed effects of treatments and cycles as well as their interaction effects on plant growth and leaf quality were analyzed using R (R Core Team 2024) and the mean comparison was conducted using Tukey's honestly significant difference test at 5% probability ($P < 0.05$) when treatment effects differ according to ANOVA test using R package *agricolae*. R packages *ggplot2* and *ggpubr* were used to create line charts for DO level changes and plant shoot and root growth performances, and significant levels were labeled based on the treatment comparison results from Tukey's honestly significant difference test.

Results

Real-time adjusted DO enrichment system. The DO enrichment treatments were mostly maintained at the target DO values with few fluctuations (Fig. 1), indicating the ability of the system to maintain a stable DO. DO levels from the control fluctuated between 6.5 and 8 mg·L^{-1}, while the stock solution could reach up to 30 mg L^{-1}. Average water pump turned-on times for DO10, DO15, and DO20 treatments per cycle during the

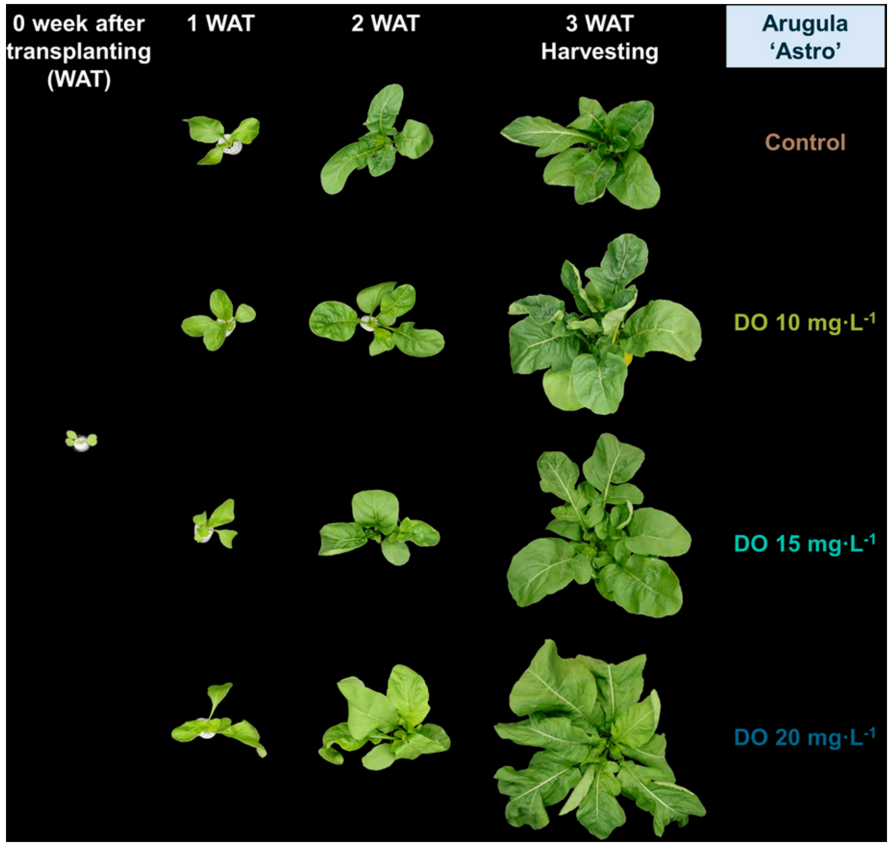

Fig. 2. Top-view photos of arugula 'Astro' shoot growth as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. WAT = week(s) after transplanting.

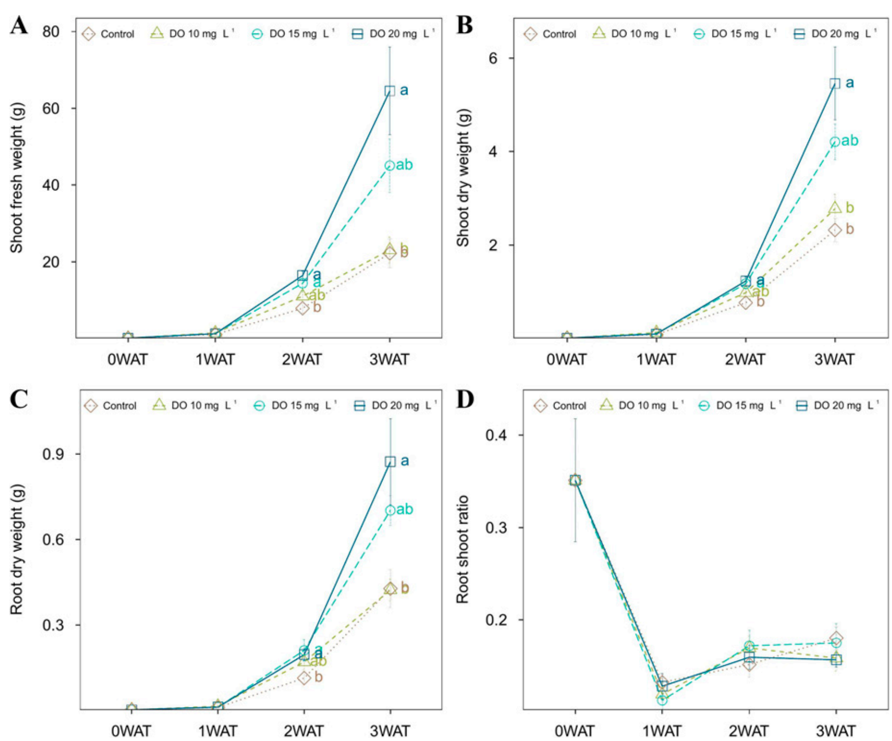

Fig. 3. Arugula 'Astro' shoot fresh weight (**A**), shoot dry weight (**B**), root dry weight (**C**), and root–shoot ratio (**D**) as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. Error bars represent the standard error (n = 3). WAT = week(s) after transplanting.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

3-week growth period were 10.9, 30.5, and 44.1 h, respectively, which led to averaged DO concentrations of 11.3, 15.8, and 20.2 mg·L^{-1}, respectively.

Effects of DO enrichment on arugula growth. DO enrichment significantly improved arugula 'Astro' growth, and this benefit was consistent across cycles as there were no significant interaction effects between DO treatments and cycles. Enhanced DO levels, especially DO15 and DO20 treatments, increased plant shoot fresh yield and dry biomass by 51% to 135% compared with control and DO10 treatments, and these improvements were shown as early as 2 weeks after transplanting (WAT). At the end of the study, compared with the control and DO10 treatments, DO20 treatment showed 191% and 180% increases in fresh yield, while DO15 improved yields by 103% and 95%, respectively (Figs. 2 and 3). Root development showed similar trends, and DO15 and DO20 treatments significantly improved root dry weight compared with the control and DO10; however, elevated DO treatments did not change the plant root–shoot ratio (Supplemental Fig. 2). Leaf greenness showed reverse effects, as higher DO level decreased the leaf greenness by 11% to 16% compared with control and DO10 treatments, possibly due to enlarged leaves having decreased chlorophyll concentration. There were no significant differences in soluble solid content and leaf net assimilation rate; however, a 45% lower transpiration rate was detected under DO10 treatment, leading to a 21% higher instantaneous WUE compared with the control. At the same time, leaf nitrogen, phosphorus, and potassium concentrations were also lower in DO 10 treatment but only during the first cycle (Table 1).

Similarly, arugula 'Esmee' shoot and root growth was significantly affected by DO treatment with consistent results across cycles, as there were no significant interaction effects between DO treatments and cycles. The beneficial effects from elevated DO levels were found later than 'Astro' at 3 WAT instead of 2 WAT, and DO15 treatment promoted a 148% improvement in plant yield with increases of dry biomass for both shoot (147%) and root (117%) as compared with control, while no significant differences were found between DO10 and control ($P > 0.05$), and elevated DO treatments did not change the plant root–shoot ratio (Figs. 4 and 5; Supplemental Fig. 3). No differences were detected due to DO enrichment in leaf gas exchange (net assimilation rate, transpiration), chlorophyll, soluble solid, and nitrogen content, while higher phosphorus and potassium concentrations were observed in DO enrichment treatments (Table 1).

Effects of DO enrichment on kale growth. Unlike arugula, enriched DO treatment had no significant effects on kale 'KX-1' or 'Red Russian' shoot and root growth ($P > 0.05$), while DO15 and DO20 treatments had numerically higher shoot fresh weight and root dry weight compared with control and DO10 (Figs. 6–9; Supplemental Figs. 4 and 5); there were no differences in leaf greenness, soluble solid content, leaf net assimilation rate,

Table 1. Effects of dissolved oxygen (DO) treatments and growth cycles on arugula 'Astro' and 'Esmee' growth, gas exchange, and nutrient content changes.

Treatments and growth cycles	LG (SPAD)	SSC (%)	*A* (μmol·m^{-2}·s^{-1})	*E* (mmol·m^{-2}·s^{-1})	N (%)	P (%)	K (%)
Astro							
DO Trt							
Control	52.77 ab	4.93	22.73	5.85 ab	6.57 a	0.67 a	6.86 a
DO10	58.00 a	5.07	17.76	3.24 b	4.99 b	0.51 b	5.08 b
DO15	48.82 b	4.23	27.07	8.38 a	6.12 a	0.60 a	6.27 a
DO20	47.15 b	4.40	26.55	5.94 ab	6.66 a	0.67 a	6.39 a
Growth cycle							
First	50.60	4.16 b	27.95 a	6.55	6.16	0.52 b	6.40
Second	52.77	5.16 a	19.11 b	5.16	6.01	0.71 a	5.89
P values							
Trt	**	NS	NS	*	***	***	***
Cycle	NS	*	*	NS	NS	***	NS
Trt × cycle	NS	NS	NS	NS	*	NS	**
Esmee							
DO Trt							
Control	50.75	4.65	12.85	3.15	5.60	0.51 b	4.82 b
DO10	52.07	5.58	16.98	4.01	5.88	0.70 a	6.69 a
DO15	56.28	5.13	17.34	3.49	5.60	0.61 ab	6.05 ab
DO20	49.38	4.52	17.95	4.06	5.63	0.60 ab	6.08 ab
Growth cycle							
First	50.23	4.76	20.87 a	4.45	5.98 a	0.47 b	6.07
Second	54.02	5.18	11.69 b	2.90	5.37 b	0.74 a	5.75
P values							
Trt	NS	NS	NS	NS	NS	***	**
Cycle	NS	NS	**	NS	***	***	NS
Trt × cycle	NS	NS	NS	NS	NS	NS	NS

Different letters within a column from the same factor indicate significant differences at α = 0.05, according to Tukey's honestly significant difference test. NS, *, **, and *** indicate nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively. LG = leaf greenness; SPAD = soil plant analysis development unit; SSC = soluble solids content; *A* = net assimilation rate; *E* = transpiration rate; N = nitrogen; P = phosphorus; K = potassium; Trt = treatment.

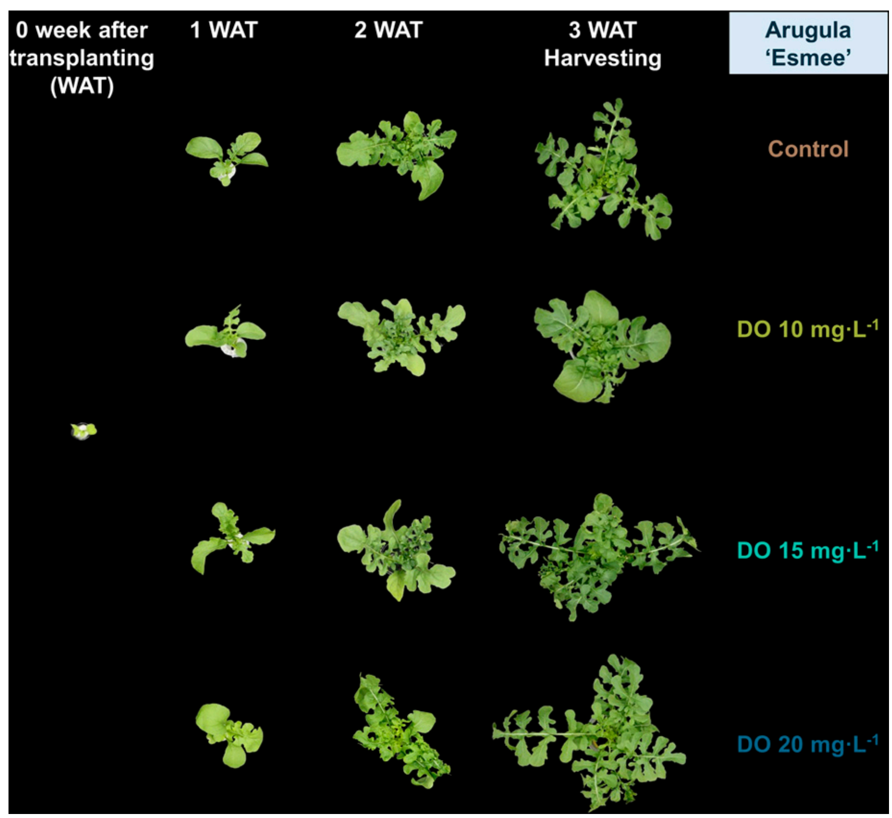

Fig. 4. Top-view photos of arugula 'Esmee' shoot growth as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. WAT = week(s) after transplanting.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

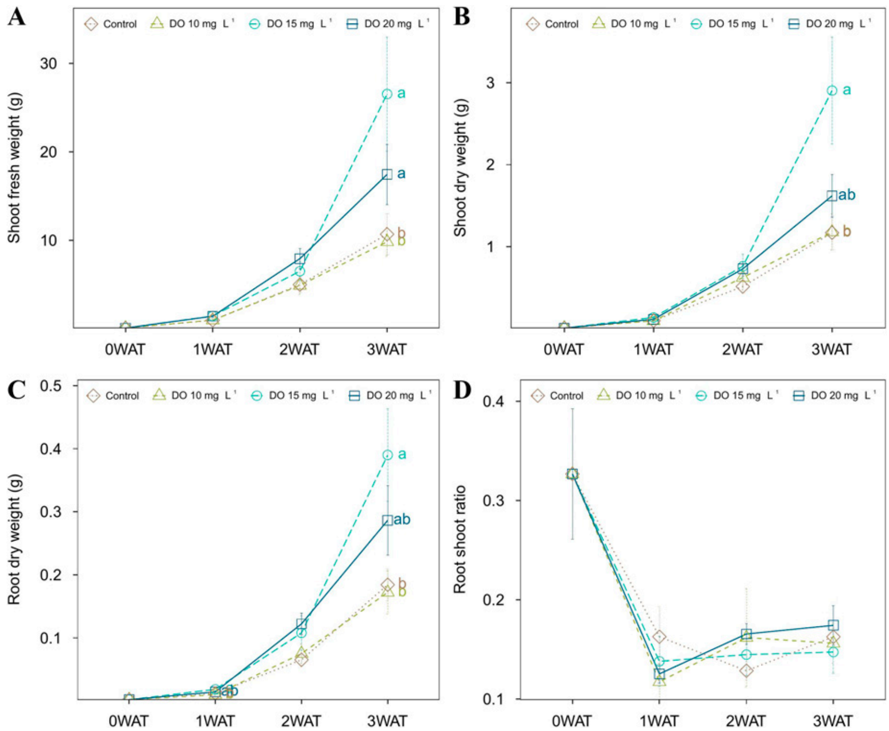

Fig. 5. Arugula 'Esmee' shoot fresh weight (**A**), shoot dry weight (**B**), root dry weight (**C**), and root–shoot ratio (**D**) as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. Error bars represent the standard error (n = 3). WAT = week(s) after transplanting.

stomatal conductance, transpiration, N and K concentrations between DO treatments regardless of the kale cultivars ($P > 0.05$), except a lower P concentration was detected in 'KX-1' under DO10 treatment ($P < 0.05$) (Table 2).

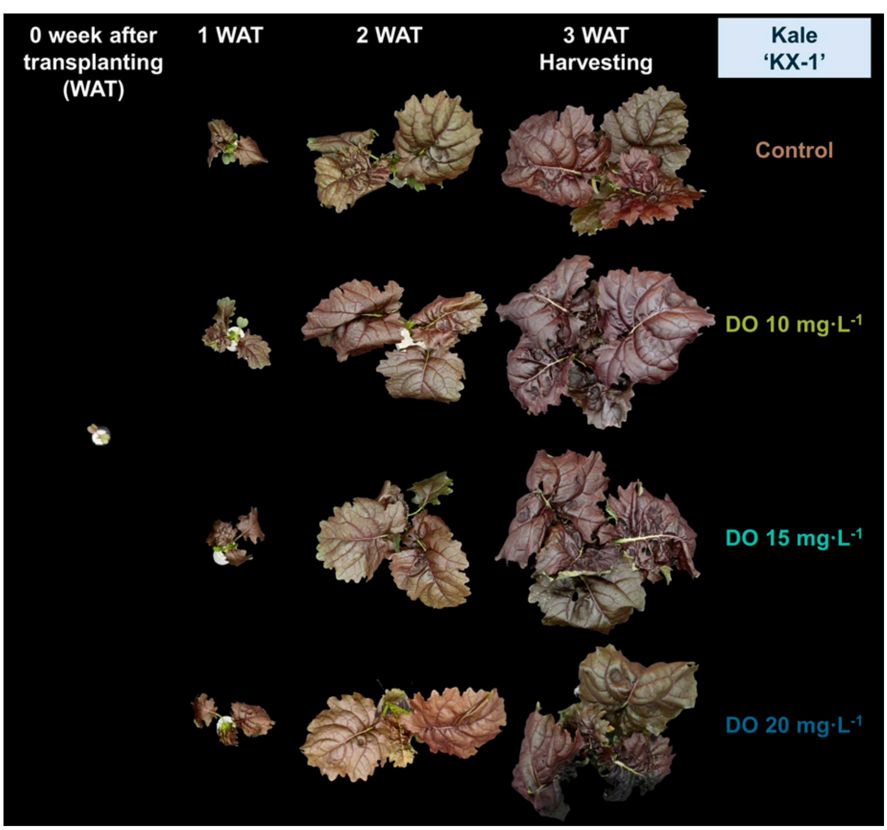

Fig. 6. Top-view photos of kale 'KX-1' shoot growth as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. WAT = week(s) after transplanting.

Energy cost and yield return. Considering all the devices used during the 21-d growth period (504 h total), that average energy cost for control was 181.4 kWh, that for DO10 was 440.6 kWh, that for DO 15 was 442.6 kWh, and that for DO 20 was 443.9 kWh. Yield return was calculated based on 108 plants (36 plants per tray in three trays). As shown in Table 3, based on the percentage, only DO15 treatment using 'Esmee' and DO20 treatment using 'Astro' could have a higher yield return compared with the extra energy cost: 148% vs. 144% and 191% vs. 145%; based on the economic analysis, only DO20 treatment using 'Astro' could have a positive net return values ($15.21 during the 21-d growth period), while all the other treatments showed reduced profit due to excessive energy cost, indicating there were no economic benefits introducing DO enrichment in these scenarios, especially not recommended for use in kale production.

Discussion

Growth cycle had consistent effects on arugula and kale growth. Air environmental conditions showed slight differences between the two cycles and the second cycle had a higher air temperature and a higher VPD compared with the first cycle, which led to higher plant transpiration. Higher temperatures enhance plant transpiration by facilitating transmembrane water movement and reducing water viscosity, while plant transpiration increases along with increasing VPD (especially under 2 kPa) due to the difference in water vapor pressure between leaf and air that drives the water evaporation (Sadok et al. 2021). The second cycle had a 15% higher DLI than the first cycle, resulting in higher fresh yields and dry biomass across all crops and cultivars. A study showed that a 1 $mol \cdot m^{-2} \cdot d^{-1}$ increase in DLI could lead to a 3 g fresh mass increase and a 0.1 g dry mass increase in lettuce, while leaf chlorophyll content was improved due to increased DLI (Kelly et al. 2020). Our study confirmed that the effects of DLI were consistent in arugula and kale.

DO enrichment had varied effects on arugula and kale growth. Despite the environmental differences, our study showed that DO treatments induced consistent effects on plant morpho-physiological responses across cycles. Multiple studies have demonstrated the benefits of elevated DO treatment on both leafy and fruiting plant growth; however, the mechanisms of enhancement may differ. Zhang et al. (2024) found that irrigating the tomato (*Solanum lycopersicum*) root zone with water containing a high DO level (15 $mg \cdot L^{-1}$) significantly increased leaf net photosynthetic rates compared with the control, which led to improved carbon assimilation in plant tissues, resulting in higher sucrose content in the roots and increased starch and sucrose levels in the leaves, and ultimately, these effects promoted plant growth, increased fruit yield, and enhanced fruit quality; however, an excessively high DO level water (30 $mg \cdot L^{-1}$) did not

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

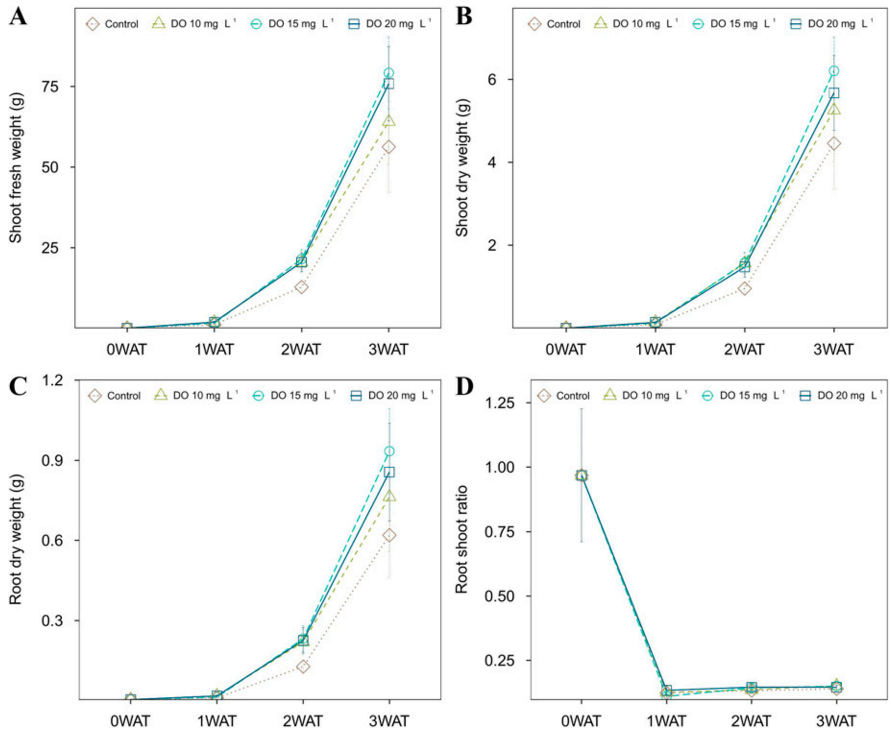

Fig. 7. Kale 'KX-1' shoot fresh weight (**A**), shoot dry weight (**B**), root dry weight (**C**), and root–shoot ratio (**D**) as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. Error bars represent the standard error (n = 3). WAT = week(s) after transplanting.

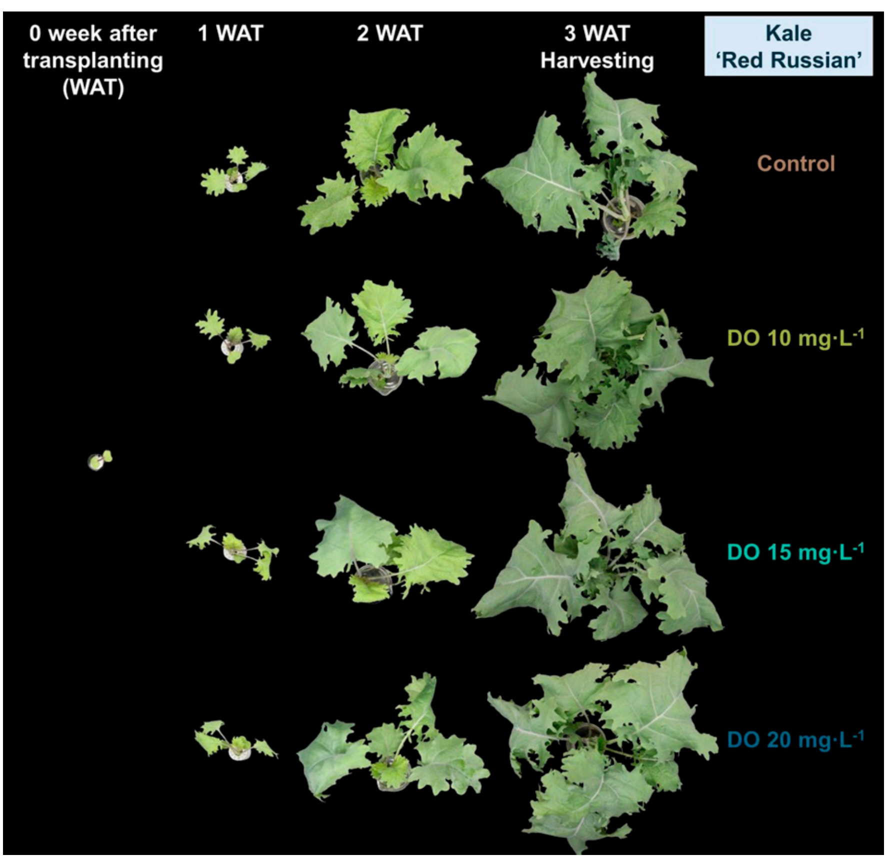

Fig. 8. Top-view photos of kale 'Red Russian' shoot growth as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. WAT = week(s) after transplanting.

provide additional benefits. Under hydroponic leafy greens, elevated DO treatment benefited plant biomass and root development; however, there were no significant effects on plant gas exchange responses (photosynthetic rate, stomatal conductance, transpiration rate) only by enhancing water DO level alone (Nitu et al. 2024). Our results also found that elevated DO levels from 10 to 20 $mg{\cdot}L^{-1}$ did not change arugula and kale gas exchange performance except for a numerical increase. Therefore, the benefits from DO enhancement were not due to the changes of the gas exchange rate; instead, positive root development with higher water and nutrient uptake caused the improved shoot growth, which eventually resulted in an unchanged root–shoot ratio.

Oxygen-rich water improved root respiration, which generated energy to support cell division and elongation, promoted lateral root development and root hair formation, and increased root surface area (Moreno Roblero et al. 2020). We found that plants grown under control had longer primary roots but less compacted lateral roots and root hairs compared with DO enrichment treatments (10 to 20 $mg{\cdot}L^{-1}$), which indicated that high DO could lead to increased root branching but decreased elongation, as the roots needed expansion to access sufficient oxygen. Although these root structure changes happened in both arugula and kale crops and cultivars, the eventual benefits were crop specific, as arugula benefited more from elevated DO levels and root development, which could economically justify the additional energy cost; while kale did not require high DO level solutions, and a standard air pump-based aeration would meet the root respiration demand. Although studies have demonstrated that DO enrichment had positive effects on the growth of hydroponically grown lettuce, microgreen, tomato, and pepper (Grishin et al. 2021; Kurashina et al. 2019; Roosta 2024; Zhao et al. 2024), our study showed that it is necessary to test other crops' adaptivity to the elevated DO, and further research is needed to understand physio-biochemical mechanisms behind these responses. Additionally, a high level of DO could cause the oxidation of iron and manganese cations to nonbioavailable forms, reducing their availability to plants. Even though we did not find significant differences in plant iron and manganese concentrations between control and DO enrichment treatments in our study, this oxidization could be an issue in longer, continuous cycles, which should be considered to test in future studies.

System consideration and future research. Traditional aeration systems, such as surface aerators and diffused aeration, are widely used due to their simplicity and low upfront cost; however, these systems usually require high energy consumption (e.g., air pump electrical power 360 W), and they have lower oxygen transfer dynamics (47% lower) comparing to micronano bubble–based oxygen enrichment techniques (Kizhisseri et al. 2025). Our study presented a real-time adjusted DO enrichment platform using oxygen generator and oxygen infusion tube–based inline recirculation system, which could improve oxygen solubility

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

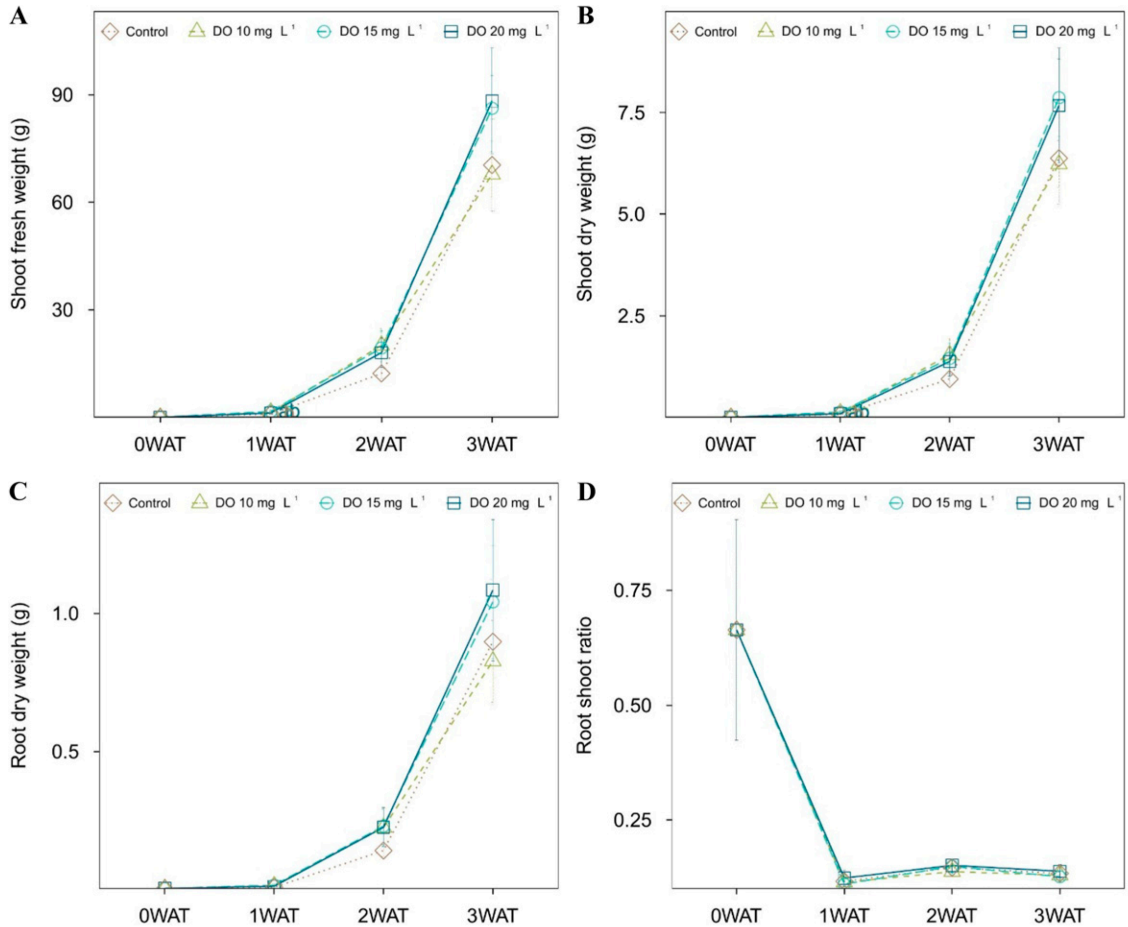

Fig. 9. Kale 'Red Russian' shoot fresh weight (**A**), shoot dry weight (**B**), root dry weight (**C**), and root–shoot ratio (**D**) as affected by control and three dissolved oxygen (DO) enrichment treatments during the study period from transplanting to harvesting. Error bars represent the standard error (n = 3). WAT = week(s) after transplanting.

Table 2. Effects of dissolved oxygen (DO) treatments and growth cycle on kale 'KX-1' and 'Red Russian' growth, gas exchange, and nutrient content changes.

Treatments and growth cycles	LG (SPAD)	SSC (%)	*A* (μmol·m^{-2}·s^{-1})	*E* (μmol·m^{-2}·s^{-1})	N (%)	P (%)	K (%)
KX-1							
DO Trt							
Control	40.25	4.05	24.87	9.21	7.03	1.04 a	6.75
DO10	43.43	3.93	23.82	7.97	6.69	0.93 b	6.28
DO15	41.57	4.15	24.71	8.61	6.98	1.02 ab	6.51
DO20	38.67	3.53	23.08	8.03	7.12	1.06 a	6.65
Growth cycle							
First	37.98 b	4.14	25.08	7.81 b	6.66 b	0.86 b	6.63
Second	43.98 a	3.69	23.15	9.11 a	7.24 a	1.17 a	6.47
P values							
Trt	NS	NS	NS	NS	NS	*	NS
Cycle	**	NS	NS	*	**	***	NS
Trt × cycle	NS	NS	NS	NS	NS	NS	NS
Red Russian							
DO Trt							
Control	41.70	4.45	28.64	9.46	6.52	0.82	6.32
DO10	43.38	4.48	26.59	8.49	6.42	0.85	6.42
DO15	43.38	4.03	28.03	8.49	6.18	0.82	6.25
DO20	42.02	4.10	27.00	7.90	6.49	0.86	6.66
Growth cycle							
First	39.88 b	4.34	27.58	7.68 b	6.13 b	0.72 b	6.46
Second	45.36 a	4.19	27.55	9.49 a	6.67 a	0.96 a	6.37
P values							
Trt	NS	NS	NS	NS	NS	NS	NS
Cycle	***	NS	NS	**	***	***	NS
Trt × cycle	NS	NS	NS	NS	NS	NS	NS

Different letters within a column from the same factor indicate significant differences at α = 0.05, according to Tukey's honestly significant difference test. NS, *, **, and *** indicate nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively. LG = leaf greenness; SPAD = soil plant analysis development unit; SSC = soluble solids content; *A* = net assimilation rate; *E* = transpiration rate; N = nitrogen; P = phosphorus; K = potassium; Trt = treatment.

by prolonging gas retention times, leading to consistent beneficial effects. Alternative DO enrichment methods existed, such as using the oxygen fertilizers hydrogen peroxide, calcium peroxide, and magnesium peroxide (Lau and Mattson 2021; Liu et al. 2022); lowering solution temperature to improve oxygen solubility (Al-Rawahy et al. 2019; Ouyang et al. 2020); applying ozone in the nutrient solution (Machuca et al. 2023); injecting pure oxygen to achieve supersaturated DO levels (Kurashina et al. 2019); and using nanobubble generator in the inline system (DeBoer et al. 2024).

Future research should explore the comparison among oxygen infusion via nanobubble or ozone technologies, root zone cooling, or oxygen fertilizers to identify strategies that will achieve similar or improved plant responses with lower energy requirements, higher resource use efficiency, and promising energy cost vs. yield return. Additionally, the combined application of DO enrichment with supplemental LED lighting or optimized nitrogen fertilization (in the form of nitrate or ammonia) was shown to enhance plant gas exchange performance, further amplifying the benefits of elevated DO (Nitu et al. 2024; Roosta 2024). Other factors, such as the use of plant growth biostimulants and the optimization of root zone temperature, also hold promise as potential targets for future research.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

Table 3. Average energy cost and yield return in percentages, cost/value, and net return from dissolved oxygen (DO) enrichment treatments as compared with the control in one growth cycle.

	Compared with control								
	DO 10 mg·L^{-1}			DO 15 mg·L^{-1}			DO 20 mg·L^{-1}		
Cost or return	Percentage (%)	Cost/value ($)	Net return ($)	Percentage (%)	Cost/value ($)	Net return ($)	Percentage (%)	Cost/value ($)	Net return ($)
Energy cost	+143	+34.73		+144%	+$35.00		+145	+35.18	
Yield return									
Astro	+4	+1.06	−33.67	+103	+27.21	−7.79	+191	+50.39	+15.21
Esmee	−8	−1.02	−35.75	+148	+18.85	−16.15	+63	+8.05	−27.13
KX-1	+14	+1.88	−32.85	+41	+5.53	−29.47	+35	+4.72	−30.46
Red Russian	−4	−0.62	−35.35	+22	+3.81	−31.19	+25	+4.30	−30.88

The percentage was calculated as (treatment − control)/control × 100%. A plus sign indicates an increase, and a minus sign indicates a decrease. Electricity cost was based on $0.134/kWh. Yield return was calculated based on 108 plants (36 plants per tray in three trays) with arugula retail prices $11.02/kg and kale retail prices $2.23/kg.

Conclusions

This two-cycle study demonstrated a successful real-time DO adjustment system that provided targeted DO levels (10, 15, and 20 mg·L^{-1}). Beneficial effects from DO enrichment were crop specific, and arugula showed significant benefits when using high DO level water (15 to 20 mg·L^{-1}) with higher biomass and nutrients, with Astro cultivar showing early benefits as soon as 2 weeks after treatments. There were no significant benefits using high DO level water for kale growth. DO enrichment had no effects on plant root to shoot ratio, plant quality, or gas exchange performances. A simple economic assessment showed that a high DO level (20 mg·L^{-1}) brought a net return of $0.14 increase per plant for arugula production. Crop-specific and economic considerations with energy cost and yield return need to be analyzed in depth before the large-scale implementation of DO enrichment in hydroponic production.

References Cited

Al-Rawahy MS, Al-Rawahy SA, Al-Mulla YA, Nadaf SK. 2019. Influence of nutrient solution temperature on its oxygen level and growth, yield and quality of hydroponic cucumber. J Agric Sci. 11(3):75–92. https://doi.org/10.5539/jas.v11n3p75.

DeBoer EJ, Richardson MD, Gentimis T, McCalla JH. 2024. Analysis of nanobubble-oxygenated water for horticultural applications. HortTechnology. 34(6):769–773. https://doi.org/10.21273/HORTTECH05524-24.

Goto E, Both AJ, Albright LD, Langhans RW, Leed AR. 1996. Effect of dissolved oxygen concentration on lettuce growth in floating hydroponics. Acta Hortic. 440:205–210. https://doi.org/10.17660/actahortic.1996.440.36.

Grishin A, Grishin A, Semenova NA, Grishin V, Knyazeva I, Dorochov A. 2021. The effect of dissolved oxygen on microgreen productivity. BIO Web Conf. 30:05002. https://doi.org/10.1051/bioconf/20213005002.

Hendrickson T, Dunn BL, Goad C, Hu B, Singh H. 2022. Effects of elevated water temperature on growth of basil using nutrient film technique. HortScience. 57(8):925–932. https://doi.org/10.21273/HORTSCI16690-22.

Kelly N, Choe D, Meng Q, Runkle ES. 2020. Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Sci Hortic. 272:109565. https://doi.org/10.1016/j.scienta.2020.109565.

Kizhisseri MI, Sakr M, Maraqa M, Mohamed MM. 2025. A comparative bench scale study of oxygen transfer dynamics using micro-nano bubbles and conventional aeration in water treatment systems. Heliyon. 11(4):e41687. https://doi.org/10.1016/j.heliyon.2025.e41687.

Kurashina Y, Yamashita T, Kurabayashi S, Takemura K, Ando K. 2019. Growth control of leaf lettuce with exposure to underwater ultrasound and dissolved oxygen supersaturation. Ultrason Sonochem. 51:292–297. https://doi.org/10.1016/j.ultsonch.2018.10.005.

Lau V, Mattson N. 2021. Effects of hydrogen peroxide on organically fertilized hydroponic lettuce (*Lactuca sativa* L.). Horticulturae. 7(5):106. https://doi.org/10.3390/horticulturae7050106.

Liu D, Paul A-L, Morgan KT, Liu G. 2022. Effects of oxygen fertilization on damage reduction in flooded snap bean (*Phaseolus vulgaris* L.). Sci Rep. 12(1):4282. https://doi.org/10.1038/s41598-022-08165-5.

Machuca VA, Silveira GAC, Hernández-Adasme C, Escalona CVH. 2023. Effect of the ozone application in the nutrient solution and the yield and oxidative stress of hydroponic baby red chard. Horticulturae. 9(11):1234. https://doi.org/10.3390/horticulturae9111234.

Moreno Roblero M, Pineda J, Colinas León MT, Sahagún Castellanos J. 2020. Oxygen in the root zone and its effect on plants. Rev Mexicana Cienc Agric. 11:931–943. https://doi.org/10.29312/remexca.v11i4.2128.

Nitu OA, Ivan EŞ, Tronac AS, Arshad A. 2024. Optimizing lettuce growth in nutrient film technique hydroponics: Evaluating the impact of elevated oxygen concentrations in the root zone under LED illumination. Agronomy. 14(9):1896. https://doi.org/10.3390/agronomy14091896.

Ouyang Z, Tian J, Yan X, Shen H. 2020. Effects of different concentrations of dissolved oxygen or temperatures on the growth, photosynthesis, yield and quality of lettuce. Agric Water Manage. 228:105896. https://doi.org/10.1016/j.agwat.2019.105896.

R Core Team. 2024. R-4.4.1 for Windows. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org. [accessed 10 Sep 2024].

Ragaveena S, Edward AS, Surendran U. 2021. Smart controlled environment agriculture methods: A holistic review. Rev Environ Sci Biotechnol. 20(4):887–913. https://doi.org/10.1007/s11157-021-09591-z.

Roosta HR. 2024. The responses of pepper plants to nitrogen form and dissolved oxygen concentration of nutrient solution in hydroponics. BMC Plant Biol. 24(1):281. https://doi.org/10.1186/s12870-024-04943-7.

Sadok W, Lopez JR, Smith KP. 2021. Transpiration increases under high-temperature stress: Potential mechanisms, trade-offs and prospects for crop resilience in a warming world. Plant Cell Environ. 44(7):2102–2116. https://doi.org/10.1111/pce.13970.

TRIDGE. 2025. https://www.tridge.com/. [accessed 16 Jul 2025].

US Department of Agriculture. 2025. Retail reports. https://www.ams.usda.gov/market-news/retail. [accessed 16 Jul 2025].

US Energy Information Administration. 2025. https://www.eia.gov/. [accessed 16 Jul 2025].

Zhang Z, Yang R, Sun J, Li Y, Geng Y, Pan Y, Zhang Z. 2024. Root-zone aeration improves fruit yield and quality of tomato by enhancement of leaf photosynthetic performance. Agric Water Manage. 291:108639. https://doi.org/10.1016/j.agwat.2023.108639.

Zhao Q, Guo S, Feng J, Li D, Yang S, Zhou X. 2024. Suitable water–fertilizer management and ozone synergy can enhance substrate-based lettuce yield and water–fertilizer use efficiency. Agronomy. 14(8):1619. https://doi.org/10.3390/agronomy14081619.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-10-01 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/