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Red:Blue ratios differently change yield, nutritional quality and water use efficiency of twelve microgreen crops

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Abstract. Microgreens are young seedlings harvested at the first true leaf stage and are valued for their concentrated flavour and phytonutrients. Their short growth cycle and high planting density make them well-suited for Totally Controlled Environment Agriculture (TCEA), which shields crop performance from external weather and water scarcity. However, species-specific water footprints for these juvenile crops remain limited, which inhibits accurate sustainability claims. This study evaluated twelve culinary microgreen species representing *Amaryllidaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Lamiaceae*, cultivated in a micro-TCEA unit under two LED spectra with contrasting Red:Blue ratios (5.08 vs 2.33; 255 µmol m^{-2} s^{-1}; 14 h photoperiod). Ranking species by days to harvest and cumulative water use produced a multidimensional efficiency matrix linking physiology to resource demands. The analysis revealed clear interspecific differences in growth duration and water consumption, which directly affected water-use efficiency (ranging from 73 to 154 g biomass L^{-1}). Spectral treatment did not alter biomass, water use efficiency, nitrate, or phenolic content, confirming nutritional parity across light regimes. The observed variability among species in crop length and water budget highlights that early-stage demand is primarily driven by intrinsic physiological traits. Adopting species-specific irrigation set points offers a high-leverage route to maximise yield while minimising water withdrawal, providing a practical framework for cultivar choice, scheduling, and climate-adaptive strategies in precise horticulture.

Key words: vertical farming, irrigation, LED spectra, phenolic profile, nitrate, controlled environment agriculture.

Introduction

Agriculture consistently absorbs approximately 70 percent of the freshwater withdrawn worldwide (Fathidarehnijeh *et al.*, 2024; Tavan *et al.*, 2021). It is estimated that the majority (87%) of freshwater is used for the primary sector, agriculture, on a global scale (Gruda, 2019). In addition, agriculture is defined as the largest consumer of fresh water because of its low water use efficiency, which is linked to the technical complexities related to the distribution and maintenance of irrigation infrastructure (Pignata, Casale, & Nicola, 2017). In some parts of the world, such as the Mediterranean basin and other semi-arid hotspots, the demand for water sources exceeds the natural recharge cycle of the water sources that flow through them, foreshadowing potential conflicts with civil and ecosystem use (Gruda, 2019). This situation generates a growing vulnerability to climate change, which intensifies droughts and irregular precipitation patterns (Fathidarehnijeh *et al.*, 2024; Gruda, 2019). Water scarcity is an acute problem in these regions, threatening to impede economic development and cause serious environmental and social problems. The aggravation caused by climate change is expected to lead to more frequent extreme events, such as prolonged droughts and uneven rainfall distribution, which can lead to reduced agricultural yields and greater yield variability (Fathidarehnijeh *et al.*, 2024). Reducing water pressure cannot be limited to linear cuts in irrigation volumes; it must involve enhancing the efficiency with which every litre is transformed into biomass or nutritional value (Alizaeh *et al.*, 2025;

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Langenfeld *et al.*, 2022). This concept underpins water use efficiency (WUE), which is defined as the amount of biomass produced per unit of water consumed or transpired (Langenfeld *et al.*, 2022; Mir & Khah, 2024). Improving WUE has direct economic and environmental impacts (Gruda, 2019).

Improvements in WUE can be achieved through soilless cultivation systems (SCS), such as hydroponics, which, in the last 10 years, are emerging as promising solutions to confront these challenges in several aspects (Nicola, Hoeberechts, & Fontana, 2007; Pignata, Casale, & Nicola, 2017):

I) Reduction in water consumption: SCS use significantly less water than traditional open-field methods (Alizaeh *et al.*, 2025; Pignata, Casale, & Nicola, 2017). In general, SCS can employ only 5-10% of the water required to cultivate the same crop in an open field. In accordance with this reduction, some studies have shown that hydroponics can reduce water consumption by a factor of 30–50 compared with open-field or greenhouse cultivation (Pennisi *et al.*, 2019; Pomoni *et al.*, 2023). For instance, it is estimated that 1 kg of lettuce produced in an indoor soilless farm requires only 3% of the water that might be needed for field-grown lettuce (O'Sullivan *et al.*, 2019).

II) Control and precision: SCS offers tight control over growth factors, including the delivery of water and nutrients, thereby improving input-use efficiency and reducing losses (Chrysargyris & Tzortzakis 2025; Fathidarehnijeh *et al.*, 2024). Water is supplied directly to plant roots in precise amounts and at the appropriate time. This precision enables optimised water management for plant growth, leading to considerable water savings relative to conventional agriculture (Alizaeh *et al.*, 2025).

III) Closed-loop systems: Closed-loop configurations, which recycle drainage water, are particularly effective at reducing water and nutrient losses, achieving up to 90% water savings and 85% nutrient savings compared with open-loop systems (Kozai, 2013). The reuse of nutrient solutions in closed-loop hydroponics lowers water consumption and enhances WUE (Massa *et al.*, 2019).

The technological framework that makes these figures possible is totally controlled environment agriculture (TCEA) and, with particular focus in recent years, multilayer vertical cultivation (Bantis, 2021; Kozai, 2013). These systems represent a promising solution for food security in regions where environmental conditions, such as high temperatures and limited water availability, constrain agricultural production (Alrajhi *et al.*, 2023). In TCEA, the water flow is closed and filtered; LED lamps replace solar radiation; sensors and artificial intelligence modulate temperature, humidity, CO_2, pH, and EC in real time; production becomes independent of the seasons (Alrajhi *et al.*, 2023; Hayashi, 2023; Pignata, Casale, & Nicola, 2017). LED lighting is the sole light source in TCEA, offering control over the spectrum, intensity, and photoperiod (Bucky *et al.*, 2024). This advanced control is important for improving plant growth and using resources efficiently in controlled environments. Changing the light spectrum, especially the Red:Blue (R:B) ratio, can affect plant responses. For example, increasing red light can improve WUE (Mir & Khah, 2024; Pennisi *et al.*, 2019). Specifically, as the red portion of the spectrum increases, plant transpiration decreases more rapidly than the quantum efficiency of Photosystem II (PSII), leading to a higher WUE. The reduction in transpiration is associated with lower stomatal conductance, which is related to a reduced stomatal density. For lettuce, for example, an 3 R:B maximised yield and WUE, reaching values of up to 75 g fresh weight per litre of water (FW L^{-1} H_2O) (Pennisi *et al.*, 2019). These values represent a substantial improvement over the 4–19 g FW L^{-1} H_2O achieved in traditional open-field cultivation or the 50 g FW L^{-1} H_2O typical of conventional hydroponic greenhouses.

Microgreens are young plants that are picked 7–21 days after planting, depending on the species. They grow well in these settings, and their growth and quality can be adjusted using special LED lights (Balik *et al.*, 2025; Bulgari *et al.*, 2017; Rizvi *et al.*, 2024). They constitute a promising resource for food and nutritional security (Di Gioia *et al.*, 2023), especially in areas with limited access to fresh vegetables or restrictive climatic conditions (Vrkić *et al.*, 2024). Beyond being a culinary novelty valued for their intense flavours, vibrant colours, and crisp textures, microgreens are known for their superior nutritional profile. They contain carotenoids, vitamin C (ascorbic acid) (Dereje *et al.*, 2023), and phenolic compounds (including flavonoids) (Alrajhi *et al.*, 2023) at concentrations often equal to or higher than those found in mature vegetables. They also exhibit low levels of anti-nutrients, such as nitrates, making them safer for consumption (Jasenovska *et al.*, 2024). Their short cycle and reduced stature render them ideal for soilless and multilayer vertical systems typical of TCEA (Bantis, 2021), allowing optimal exploitation of vertical space and extremely rapid crop rotations, thereby maximising production per unit area (Vrkić *et al.*, 2024). Customisable LED lighting and sensor- and AI-based monitoring further optimise input efficiency and the modulation of microgreens growth conditions

(Alrajhi *et al.*, 2023). However, species-specific data on WUE, yield, and quality of microgreens under TCEA conditions remain fragmented (Alizaeh *et al.*, 2025; Seth *et al.*, 2025). This knowledge gap hampers the formulation of robust guidelines for optimal water and nutritional sustainability (Moraru, Rusu & Mintas, 2022). To address it, the present study evaluated twelve culinary microgreen species drawn from *Amaryllidaceae, Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae* and *Lamiaceae*, cultivated in a micro-TCEA unit under two LED spectra with contrasting R:B ratios.

Material and Methods

Plant growth and experimental design

The experiment was conducted at the Department of Agricultural, Forest and Food Sciences (DISAFA) at the University of Turin in Grugliasco, Italy (45°03′59.73″N, 7°35′24.72″E), from 18 April to 6 May 2023. This study assessed twelve culinary microgreen species from the *Amaryllidaceae*, *Asteraceae*, *Brassicaceae*, *Cucurbitaceae*, *Fabaceae*, and *Lamiaceae* families, as reported in Table 1. These species were cultivated using a floating system (FS) in a Micro-TCEA unit named "Radix", which was manufactured by Sananbio (Xiamen, Fujian, China). The facility was equipped with Sananbio LED lamps offering two light spectra: a Red:Blue (R:B) ratio of 5.08, consisting of 61% red, 12% blue, 15% green, and 12% far-red, and an R:B ratio of 2.33, comprising 56% red, 24% blue, 9% green, and 11% far-red. The photosynthetic photon flux density (PPFD) was set at 255 µmol m^{-2} s^{-1}, with a 14-hour photoperiod from 6 a.m. to 8 p.m. (Daily Light Integral of 12.65 mol m^{-2} d^{-1}). The distance between each cultivation layer and the luminaries was maintained at 30 cm.

The Micro-TCEA system was fully automated using the NIDO ONE V1 hydroponic controller (Nido S.R.L., Carpineti, RE, Italy), which continuously monitored key environmental parameters, such as temperature and relative humidity. Throughout the cultivation period, environmental conditions were kept within optimal ranges, with the mean air temperature, relative humidity, and vapour pressure deficit recorded at 69%, 24.8°C, and 0.85 kPa, respectively. The nutrient solution (NS) was a modification on that used by Pignata *et al.*, (2022), consisting of a 60/40 $N\text{-}NO_3^-/N\text{-}NH_4^+$ ratio and containing (in mM): 6 N, 1 P, 3 K, 1 Mg, and 1.25 Ca. Micronutrients were provided as follows (in µM): 0.14 B, 0.05 Cu, 0.11 Fe, 0.22 Mn, 0.002 Mo, and 0.14 Zn. Lysodin® Multimix (Intrachem Production S.r.l., Grassobbio, BG, Italy) was added at a concentration of 0.30 g L^{-1}. The pH and EC of the NS were manually monitored daily and maintained between 5.5 and 6.0 pH, with an average Electrical conductivity (EC) value across the four treatments of 1.2 dS m^{-1}.

Seeds were obtained from the "Bertolino Garden" (Moncalieri, Torino, Italy). On 18 April the seeds were evenly sown on jute mats (Blue Plug 0.20 × 0.20 m, Grodan®, Rockwool B.V., Roermond, The Netherlands) and placed in square plastic containers (0.20 × 0.20 m; Xiamen, Fujian, China) consisting of two layers. The upper layer was perforated to allow the roots to pass through and to support the jute substrate, while also ensuring drainage of excess water and maintaining adequate moisture. The lower layer was sealed and served as a reservoir, designed to retain a constant depth of 3 cm of nutrient solution, corresponding to a volume of 500 cm^3, which was maintained from the first irrigation onwards. Twelve species of seeds germinated within 4–6 days at 23°C and 95% relative humidity in the dark. From 22 April to 24 (Figure 1), the plastic containers were moved to the "Radix" growth Micro-TCEA system, where they were kept under controlled light conditions for 9–13 days until harvest day, depending on the species (Figure 1). Throughout the cultivation period, the water volume was monitored, and the plants were irrigated daily. The plants were harvested 14 to 17 days after sowing (2 May to 6 May), once each species reached the first true leaf stage, which is a key parameter.

Clustering and Timing

Throughout the cultivation process, key phases, such as cotyledonary distension, emergence of the first true leaf, and harvest, were recorded for microgreens from the time of sowing (Table 1). This temporal monitoring allowed the determination of species-specific growth durations, highlighting differences in developmental rate and resource demand among crops. To determine the appropriate time to begin watering (water phase), we waited until the plants had completed their dark phase, with cotyledons fully extended and roots emerging through the jute substrate. Conversely, the application of the nutrient solution started when the roots of each species reached halfway down the tray's depth containing the water (1.5 cm). This facilitated proper root development, enabling the plant to endure any potential stress caused by the increased EC when the nutrient solution was added.

Yield, water irrigation volume and water use efficiency

Microgreens (composed by cotyledons and the first true leaf) were harvested from each replicate to measure yield (FW), recorded in g m^{-2}. 10 g of subsample of microgreens was selected from the centre of each experimental unit (plastic container) within each replicate and treatment. This subsample was immediately frozen in liquid nitrogen, grinded and stored at –80°C for subsequent physiological

Table 1

Days after sowing required for cotyledonary distension, first true leaf emergence, and harvest in 12 microgreen species, listed in ascending order of total growth duration (days after sowing)

Species	Cotyledonary distension	First true leaf	Harvest
Lactuca sativa L. 'Gentilina' (Lettuce)	5	12	17
Ocimum basilicum L. 'Fine verde' (Green basil)	3	15	17
Ocimum basilicum L. 'Red rubin' (Red basil)	4	15	17
Cichorium endivia var. *crispum* L. 'Pancalieri' (Curly endive)	5	14	16
Cichorium endivia var. *latifolium* L. 'Gigante degli ortolani' (Escarole)	6	13	16
Brassica oleracea L. 'Mercato Copenaghen' (Cabbage)	6	10	15
Brassica rapa subsp. *rapa* L. L. '50 giorni' (Turnip)	6	11	15
Allium cepa L. 'Borettana' (Onion)	9	/	14
Allium porrum L. 'Porro lungo della riviera' (Leek)	7	/	14
Cucumis melo L. 'Cantalupo de charentais' (Melon)	8	12	14
Pisum sativum L. 'Maestro' (Pea)	7	9	14
Raphanus sativus L. 'Multicolor' (Radish)	6	10	13

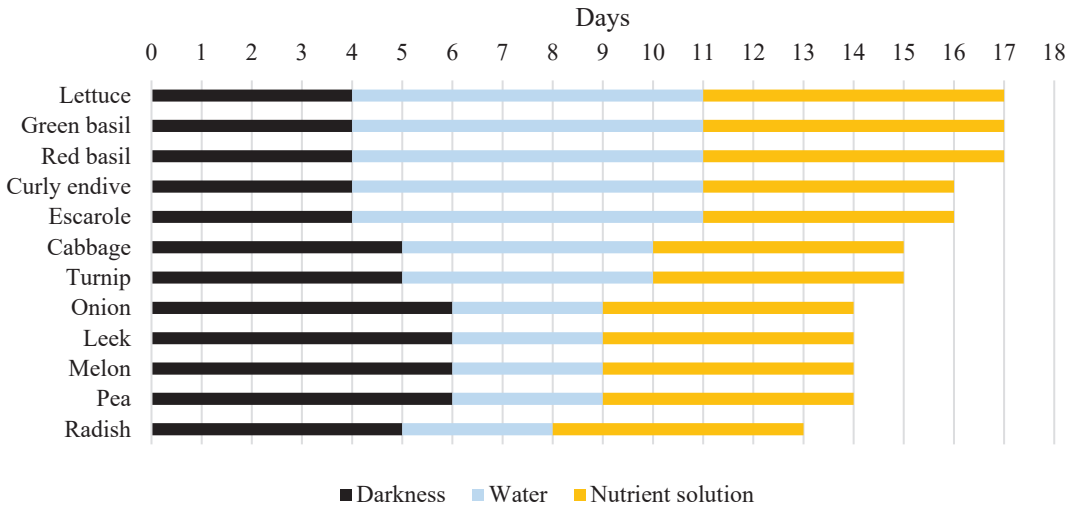

Figure 1. Timeline of dark, water-only, and nutrient-solution phases (days after sowing) for the twelve microgreen species. Black, blue, and yellow represent darkness, water irrigation, and nutrient solution irrigation, respectively.

and biochemical analyses. To determine dry weight (DW), fresh samples of varying masses were oven-dried (Binder Gesellschaft mit beschränkter Haftung, Tuttlingen, Germany (model ED56)) at 60°C until a constant weight was achieved. The daily water irrigation volume (Water) was measured using a graduated laboratory beaker and used to fill completely the second layer of cultivation container. At the end of cultivation, each water volume was related to a surface area of one square meter of cultivation. The WUE index was calculated by relating the parameters FW (g m^{-2}) and Water (L m^{-2}).

Chlorophyll content

Chlorophyll content (Chl) was analysed by grinding fresh microgreens (300 mg), per replicate, in liquid nitrogen to ensure complete cellular disruption. The ground tissue was mixed with 15 mL of high-purity ethanol (96% v/v) and stored

in complete darkness at 4°C for a controlled 24-hour extraction period. The extracts were filtered and analysed spectrophotometrically using a Cary spectrophotometre (Agilent Technologies, Santa Clara, CA, USA) at wavelengths of 665 nm for chlorophyll a (Chl a) and 649 nm for chlorophyll b (Chl b). Chl a and Chl b concentrations were calculated using the formulas from Lichtenthaler & Wellburn (1983) and expressed as mg g^{-1} of FW.

Extraction and measurement of total phenol content

Fresh grinded microgreens (1 g per replicate) were crushed in a mortar and extracted with methanol (1:3, w/v) and liquid nitrogen. The extracts were kept in an ice bath for 30 minutes and then centrifuged at 5000 g for 30 minutes at 4°C. The supernatant was stored at -20°C until analysis. The total phenol content (TPC) was measured using a modified version of the method described by Arnaldos *et al.*, (2002). To 100 µL of phenolic extract, 1 mL of 2% sodium carbonate (Na_2CO_3), and 75 µL of Folin-Ciocalteau reagent (Sigma-Aldrich, Darmstadt, Germany) were added. After incubation for 15 min at 25°C in the dark, the absorbance was measured spectrophotometrically (Cary spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 725 nm. Gallic acid was used as the standard (Meenakshi & Gnanambigai 2009).

Nitrate content

The nitrate (Nitrate) content of microgreens was determined using the salicylsulfuric method (Cataldo *et al.*, 1975). Extraction samples (20 µL per replicate) were added to 80 µL of 5% salicylic acid in sulphuric acid to 3 mL of 1.5 M NaOH. The samples were cooled at room temperature for 15 min, and spectrophotometer readings were obtained at 410 nm. Nitrate was calculated using a KNO_3 standard calibration curve (0, 1, 2.5, 5, 7.5, and 10 mM KNO_3).

Statistical Analysis

A one-way ANOVA was conducted separately for each microgreen species to evaluate the effect of the R:B light ratio on growth and quality parameters. The trials employed a randomised complete block design (RCBD) with two replicates (each replicate was composed by two repetitions, and each repetition was composed of a homogenous amount of plant sample). Mean comparisons were conducted using the Bonferroni post hoc test ($p \leq 0.05$) the results are presented as mean ± standard error (SE). ANOVA was ran using R Studio (version 2023.12.1).

Results

Yield, water irrigation volume and water use efficiency

The ANOVA indicated that altering the R:B ratio of the LED spectrum from 5.08 to 2.33 elicited species-specific responses in both FW and WUE. A significant impact on FW was observed in a subset of the 12 microgreens, whereas WUE was affected in seven species, with no consistent pattern across taxa (Table 2). The DW and Water parameters did not differ significantly among the 12 species. Regarding yield, species with longer growth cycles, such as lettuce, basil, and chicories, showed distinct sensitivities to spectral composition compared with faster-growing crops like radish, pea, or melon. Lettuce exhibited the most pronounced decline in FW under the 2.33 R:B spectrum, producing 37% less biomass compared with 5.08 R:B spectrum. Green basil also showed a 16% reduction in FW under 2.33 R:B spectrum, whereas red basil increased its FW by 43% under the same conditions compared with R:B = 5.08. Curly endive and escarole showed no significant differences between the two spectral treatments.

Within the *Brassicaceae*, radish yield increased by approximately 12% under 2.33 R:B spectrum compared 5.08 R:B spectrum, while cabbage and turnip displayed non-significant variations. Among the shorter-cycle species, melon exhibited a 15% FW reduction under 2.33 R:B spectrum compared with the 5.08 R:B spectrum, whereas onion, leek, and pea maintained comparable biomass under both spectral conditions.

As for WUE, interspecific variability was evident despite comparable irrigation volumes. Under 2.33 R:B spectrum, WUE decreased compared with 5.08 R:B spectrum in curly endive (–37%), lettuce (–35%), and green basil (–34%), while red basil exhibited a 36% increase under the same conditions. Escarole remained unaffected by spectral composition. Among the *Brassicaceae*, turnip showed higher efficiency under 5.08 R:B (31%), whereas radish displayed a moderate improvement (+14%) under the 2.33 R:B spectrum. For onion, leek, melon, and pea, WUE values remained stable between the two spectral treatments, suggesting that spectral composition exerted limited influence on species with shorter growth durations and smaller canopy size.

Chlorophyll, total phenol and nitrate content

The ANOVA revealed that modifications in the R:B ratio of the LED spectrum (5.08 vs 2.33) resulted in distinct species- and parameter-specific variations in Chl, TPC, and nitrate content. Among the 12 microgreen species examined, only two exhibited differences in Chl, two in TPC, and three in nitrate content, indicating a lack of uniform response across taxa (Table 3).

Chl only a few species showed significant responses to light quality. Escarole and pea displayed a 32% and 44 % reduction in Chl under 2.33 R:B compared to 5.08 R:B spectrum, while lettuce, basil, radish, and most of the remaining crops maintained stable chlorophyll levels. These results confirm that Chl synthesis and accumulation under controlled environments are primarily species-dependent rather than spectrum-driven.

The TPC responses were also highly variable. Most species showed no statistically significant differences between spectra, though leek recorded a 32% increase under 2.33 R:B spectrum and pea exhibited an 18% decrease compared 5.08 R:B spectrum.

Nitrate concentration, a limited number of species showed notable differences. Lettuce, red basil, and radish exhibited lower nitrate levels under 2.33 R:B spectrum (reductions of 24%, 14%, and 20%, respectively), compared 5.08 R:B, whereas the other crops showed no significant changes.

Table 2

Effects of LED light spectra on fresh weight (FW), Dry weight (DW), water irrigation volume (Water), and Water use efficiency (WUE) in 12 microgreen species

		FW	DW	Water	WUE
Species	Light (R:B)	g m-²		L m^{-2}	g L^{-1}
Lettuce	*5.08*	363.50±13.07 a	14.30±1.94 a	4.64±0.33 a	78.33±0.28 a
	2.33	227.46±2.26 b	19.75±1.42 a	4.48±0.33 a	50.81±0.96 b
	Significance	**	ns	ns	**
Green Basil	*5.08*	624.53±11.03 a	33.91±5.33 a	4.08±0.47 a	153.80±2.57 a
	2.33	522.00±2.70 a	24.62±5.32 a	5.12±0.41 a	102.20±1.93 b
	Significance	*	ns	ns	*
Red basil	*5.08*	482.56±32.48 b	17.50±4.97 a	4.72±0.41 a	102.15±1.09 b
	2.33	687.92±6.70 a	32.86±5.78 a	4.96±0.33 a	138.79±1.48 a
	Significance	*	ns	ns	**
Curly endivie	*5.08*	426.63±16.43 a	24.65±2.45 a	4.16±0.53 a	103.13±2.05 a
	2.33	381.43±59.28 a	19.23±9.36 a	5.80±0.55 a	65.36±1.92 b
	Significance	ns	ns	ns	*
Escarole	*5.08*	558.66±49.13 a	22.16±0.61 a	5.08±0.29 a	109.80±2.23 a
	2.33	445.43±172.66 a	19.38±10.18 a	4.56±0.75 a	93.93±3.88 a
	Significance	ns	ns	ns	ns
Cabbage	*5.08*	1251.01±13.08 a	182.51±66.58 a	10.88±0.62 a	115.22±1.82 a
	2.33	1147.32±111.42 a	256.00±12.22 a	9.32±0.69 a	122.88±1.45 a
	Significance	ns	ns	ns	ns
Turnip	*5.08*	737.43±107.61 a	209.58±10.96 a	9.32±0.90 a	78.90±1.10 a
	2.33	584.05±68.09 a	189.82±3.12 a	10.76±0.50 a	54.12±1.78 b
	Significance	ns	ns	ns	*
Onion	*5.08*	781.11±122.38 a	77.18±9.93 a	14.36±0.96 a	54.06±1.58 a
	2.33	695.53±54.03 a	80.90±3.65 a	15.96±0.29 a	43.55±1.47 a
	Significance	ns	ns	ns	ns
Leek	*5.08*	783.98±58.11 a	80.43±5.68 a	18.16±0.95 a	43.15±0.33 a
	2.33	748.05±23.67 a	80.61±1.33 a	15.80±0.16 a	47.34±1.06 a
	Significance	ns	ns	ns	ns
Melon	*5.08*	1722.80±5.85 a	140.77±6.12 a	15.84±0.33 a	108.76±0.71 a
	2.33	1471.31±34.03 b	128.98±3.98 a	15.92±0.33 a	92.40±0.92 b
	Significance	*	ns	ns	**
Pea	*5.08*	1667.75±243.30 a	159.31±18.91 a	18.36±0.87 a	90.36±2.36 a
	2.33	1828.26±302.01 a	179.77±19.69 a	17.20±0.78 a	105.67±2.93 a
	Significance	ns	ns	ns	ns
Radish	*5.08*	2697.93±43.81 b	347.53±16.78 a	10.40±0.53 a	259.63±2.02 b
	2.33	3012.11±6.53 a	370.63±8.41 a	10.20±0.29 a	295.33±1.41 a
	Significance	*	ns	ns	*

Different letters within columns and rows indicate statistically significant differences ($p \leq 0.05$) among light treatments: R:B 5.08 and 2.33. Data are presented as mean ± standard error (SE).
*Significance levels: *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$, and ns = not significant.*

Table 3

Effects of LED light spectra on total chlorophyll (Chl), total phenol content (TPC), and nitrate content (Nitrate) in 12 microgreen species

		Chl	TPC	Nitrate
Species	Light (R:B)	mg g^{-1} FW	mg GE g^{-1} FW	mg KNO_3 kg^{-1} (FW)
Lettuce	*5.08*	0.54±0.04 a	8.24±1.12 a	258.03±1.72 a
	2.33	0.57±0.07 a	10.04±0.01 a	196.72±2.44 b
	Significance	ns	ns	*
Green Basil	*5.08*	1.03±0.12 a	30.02±0.49 a	236.92±17.66 a
	2.33	1.38±0.14 a	29.31±0.83 a	249.99±14.07 a
	Significance	ns	ns	ns
Red basil	*5.08*	1.08±0.10 a	40.22±3.14 a	244.82±1.72 a
	2.33	0.97±0.10 a	35.92±2.23 a	211.80±4.59 b
	Significance	ns	ns	*
Curly endivie	*5.08*	1.14±0.01 a	10.14±0.75 a	211.80±4.02 a
	2.33	1.25±0.02 a	9.10±1.83 a	198.01±2.87 a
	Significance	ns	ns	ns
Escarole	*5.08*	0.76±0.03 a	9.05±0.70 a	222.28±0.14 a
	2.33	0.52±0.03 b	9.15±0.07 a	229.31±11.19 a
	Significance	*	ns	ns
Cabbage	*5.08*	1.02±0.03 a	32.38±1.57 a	215.39±0.43 a
	2.33	0.86±0.07 a	27.83±0.06 a	203.76±16.65 a
	Significance	ns	ns	ns
Turnip	*5.08*	0.63±0.12 a	21.91±1.81 a	184.23±6.60 a
	2.33	0.93±0.11 a	32.62±1.89 a	192.56±2.01 a
	Significance	ns	ns	ns
Onion	*5.08*	0.96±0.13 a	10.61±0.10 a	237.86±13.48 a
	2.33	0.91±0.01 a	10.50±1.51 a	211.24±6.36 a
	Significance	ns	ns	ns
Leek	*5.08*	1.10±0.02 a	11.73±0.27 b	234.18±9.34 a
	2.33	1.19±0.14 a	15.50±0.17 a	251.99±15.75 a
	Significance	ns	*	ns
Melon	*5.08*	1.00±0.11 a	12.09±1.64 a	229.67±11.27 a
	2.33	1.48±0.08 a	11.49±0.21 a	243.10±9.10 a
	Significance	ns	ns	ns
Pea	*5.08*	1.20±0.10 a	15.91±0.24 b	224.72±2.67 a
	2.33	0.67±0.00 b	12.97±0.32 a	238.83±3.31 a
	Significance	*	*	ns
Radish	*5.08*	0.68±0.07 a	23.62±3.11 a	184.37±3.58 b
	2.33	1.00±0.08 a	30.55±1.60 a	229.89±1.72 a
	Significance	ns	ns	*

Different letters within columns and rows indicate statistically significant differences ($p \leq 0.05$) among light treatments: R:B 5.08 and 2.33. Data are presented as mean ± standard error (SE).
*Significance levels: *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$, and ns = not significant.*

Discussion

The results obtained underscore species-specific responses to LED lighting, without an evident taxonomic trend, corroborating extensive observations in the literature that plant responses to light vary significantly depending on plant characteristics and microgreen species (Alrajhi *et al.*, 2023; Brazaitytė *et al.*, 2021; Ying *et al.*, 2020). These differences in responses are attributed to genetic variations that influence pigment composition, photoreceptors, and the morphological structure of plants (Alrajhi *et al.*, 2023). Among the leafy species with longer growth cycles, the enrichment of the blue component in the spectrum (2.33 R:B) led to a reduction in FW in lettuce (-37%) and green basil (-16%). This finding aligns with previous studies on lettuce, where an increase in the percentage of blue light resulted in a decrease in FW and DW (Clavijo-Herrera, Van Santen, & Gómez 2018; Ying *et al.*, 2020), and in basil, where high fractions of blue light can reduce fresh and dry mass (Brazaitytė *et al.*, 2016, 2021; Pennisi *et al.*, 2019). Conversely, red basil exhibited an increase in biomass (+43%) under the same enriched 2.33 R:B ratio. This positive response of basil to blue light in terms of FW has also been documented in other studies (Lobiuc *et al.*, 2017), with some basil cultivars demonstrating better growth under monochromatic or blue-enriched light (Jasenovska *et al.*, 2024).

The WUE in lettuce, endive, and green basil grown under 2.33 R:B declines significantly, with reductions ranging from -34% to -37%. This reduction aligns with the propensity of blue light to enhance stomatal aperture and conductance (Clavijo-Herrera, Van Santen, & Gómez 2018; Lim & Kim 2021; Lobiuc *et al.*, 2017), which, although it may augment CO_2 assimilation, frequently incurs a cost in terms of water efficiency (Lobiuc *et al.*, 2017). An exception of note was found in red basil cultivated under 2.33 R:B, which showed a 36% increase in WUE. This divergent response may be attributed to the substantial presence of anthocyanins in pigmented varieties (Teliban *et al.*, 2025). Anthocyanins, whose accumulation is promoted by blue light (Stamford *et al.*, 2023), function as photoprotectors by dissipating excess light energy and mitigating photoinhibition (Craver *et al.*, 2017; Pennisi *et al.*, 2019). This may lead to an overall optimisation of photosynthetic efficiency that offsets the increase in stomatal conductance, culminating in a net enhancement of WUE under conditions of elevated light.

Within the *Brassicaceae* species, only radish exhibited a positive response to the 2.33 R:B spectrum, with increases of 12% in FW and 14% in WUE. However, previous research has documented variable responses of radish to blue light, including reductions in hypocotyl length and leaf area (Ying *et al.*, 2020). Conversely, turnip demonstrated a favourable response to a red-dominant light spectrum (5.08 R:B), with a 31% increase in WUE. This finding aligns with the general trend that an increased fraction of red light enhances biomass production (Mir *et al.*, 2024; Stamford *et al.*, 2023) and potentially improves WUE. In melon experienced an approximate 15% reduction in both FW and WUE under 2.33 R:B, a pattern also observed in cucumber, a related species, where increased blue light reduced both FW and DW (Brazaitytė *et al.*, 2021; Son & Oh, 2013) and inhibited hypocotyl elongation (Hernández & Kubota 2016). In contrast, onion, leek, and pea exhibited no significant differences (Jasenovska *et al.*, 2024), further emphasising the species-specific nature of these responses (Ying *et al.*, 2020). These findings highlight the crucial role of LED light spectral composition in influencing the growth, morphology, and nutritional quality of microgreens, with effects differing by species and cultivar (Jasenovska *et al.*, 2024; Vrkić *et al.*, 2024). However, in our study, the absence of significant differences observed among some species or light treatments can be attributed to the short growth cycle of microgreens and the small spectral differences between the two R:B ratios used. According to our findings have been reported in microgreen studies where narrow R:B contrasts failed to induce significant pigment or biomass differences, suggesting that both the exposure duration and the magnitude of spectral differences are critical for eliciting measurable responses (Craver *et al.*, 2017; Ying *et al.*, 2020). The brief growth cycle typical of microgreens may have limited the exposure time needed for light spectrum differences to produce measurable effects on growth or metabolic activity (Bantis, 2021). In such short cultivation periods, spectral effects are often expressed through photomorphogenic rather than photosynthetic pathways, and the limited irradiation duration may not allow these differences to become statistically significant. The spectral combinations tested showed relatively small variations in R:B ratio, which may have been insufficient to trigger substantial physiological or biochemical changes (Orlando *et al.*, 2022).

In the ongoing analysis of species-specific responses to LED lighting, the results indicated that variations in total chlorophyll content were limited and species-dependent (Alrajhi *et al.*, 2023; Di Gioia *et al.*, 2023). Concerning the R:B ratio, red light is essential for the development of the photosynthetic apparatus and, in some cases, has shown the greatest effect on total chlorophyll accumulation (Dereje *et al.*, 2023). In contrast, blue light is recognised as crucial for chloroplast development and chlorophyll increase,

with studies confirming its positive effect on mineral accumulation through the promotion of ion transport. Additionally, a higher proportion of blue light in RB-LED lighting has been associated with increased chlorophyll content in some species (Brazaitytė *et al.*, 2021). The marked variability in responses is often attributed to factors such as genetic differences between cultivars and specific experimental conditions employed (Di Gioia *et al.*, 2023). The literature presents conflicting data regarding the influence of the light spectrum on pigments, generally suggesting a marginal effect of the spectrum on pigment levels, consistent with studies attributing a more pronounced influence to PPFD rather than R:B ratio alone (Brazaitytė *et al.*, 2015; Craver *et al.*, 2017; Frąszczak *et al.*, 2023; Kopsell *et al.*, 2012). Generally, chlorophyll and carotenoid concentrations tend to increase with rising PPFD levels (Flores *et al.*, 2024; Harakotr *et al.*, 2025). However, studies have reported conflicting results, with lower total chlorophyll concentrations under increasing light intensities or no significant effect on certain microgreens, indicating a dependence on species and cultivar (Craver *et al.*, 2017; Meas, Luengwilai, & Thongket, 2020). PPFD has also been identified as the primary factor influencing pigment accumulation (Ouzounis *et al.*, 2014). Therefore, the optimal management of LED lighting for microgreens necessitates an approach that considers both PPFD and light spectrum, acknowledging the considerable interspecific and inter-cultivar variability and the complexity of the interactions between different factors.

Research on the influence of the light spectrum on TPC in microgreens has yielded variable and species-specific outcomes (Dereje *et al.*, 2023; Harakotr *et al.*, 2025). Although the source text suggests specific findings for leek and pea, the cited sources do not provide precise data supporting a 32% increase in TPC in leek with an R:B ratio of 2.33, nor an 18% reduction in pea concerning TPC. However, it has been observed in several microgreens in this study, in accordance with pea sprouts, as noted in Bantis study (2021), that the TPC was lower under an R:B ratio of 2 compared to higher R:B ratios (5 R:B and 9 R:B), suggesting a complex dependence on the light spectrum. Consistent with its recognised phytochemical properties, red basil has demonstrated a high TPC, which can be significantly enhanced under modulated light spectra, including blue, red, and UV light, particularly when combined with fertilisation (Fayezizadeh *et al.*, 2024; Lobiuc *et al.*, 2017; Teliban *et al.*, 2025). Some studies have indicated that specific red basil cultivars exhibit maximum phenolic accumulation with higher blue light ratios (0 R:B and 0.5 R:B), whereas green basil is more stimulated by a higher proportion of red light (2 R:B) (Lobiuc *et al.*, 2017). Notably, the Ablagh genotype of basil recorded the highest levels of TPC under continuous blue light (Fayezizadeh *et al.*, 2024). The absence of evident systematic patterns in many species supports the hypothesis that TPC in microgreens is more likely induced by light stress conditions or nutritional stress rather than specific R:B ratios (Bantis, 2021). Plants produce antioxidant compounds, such as phenols, in response to environmental stressors, such as nutrient deficiency, which can alter plant metabolism and divert carbon towards the production of phenolic compounds (Keutgen *et al.*, 2021). Along with genetic differences between cultivars and specific experimental conditions (PPFD, temperature, nutrient availability), these factors are considered the primary reasons for the discrepancies observed in studies on the impact of light on phenolic production in microgreens.

Nitrate analysis indicated a substantial reduction in the three species under a 2.33 R:B: lettuce (-24%), red basil (-14%), and radish (-20%). Increased blue light content is known to stimulate nitrate reductase activity, thereby decreasing NO_3^- accumulation, a phenomenon previously documented in several baby-leaf *Brassicaceae* (Chen *et al.*, 2014; Fan *et al.*, 2019; Lobiuc *et al.*, 2017). Light is generally recognised as a key environmental factor influencing the nitrate content in vegetables by affecting the activity of the enzyme nitrate reductase (NR), which is activated by light and results in reduced nitrate accumulation (El Haddaji *et al.*, 2023; Pignata, Casale, & Nicola, 2017). Red light can lower nitrate levels in plants, such as rocket, by boosting nitrate reductase activity through phytochromes (Fayezizadeh *et al.*, 2024; Kyriacou *et al.*, 2019). More blue light in blue-red lighting also helps reduce nitrates in microgreens (Brazaitytė *et al.*, 2021). Studies on basil have shown that both blue and red light can lower nitrate content more than white light (El Haddaji *et al.*, 2023). In basil, particularly the Ablagh genotype, blue light can enhance plant nitrogen content and nitrate accumulation in the leaves, which serve as a nitrogen reserve, although levels remain below the maximum limits established by the European Commission's regulations for some leafy vegetables (EFSA CONTAM Panel, 2011; Fayezizadeh *et al.*, 2024). Variability in responses is often attributed not only to genetic differences among cultivars and species but also to specific experimental conditions, such as light intensity (PPFD), temperature, and nutrient availability, including nutrient solutions (Balik *et al.*, 2025; Stamford *et al.*, 2023). The existing gaps in understanding responses to a wide range of R:B ratios necessitate further research to optimise lighting conditions, intensity, and stress elicitors.

Conclusions

This study aimed to investigate whether altering R:B ratios balance of LED light from a red-dominant spectrum to one enriched with blue light could change the yield, water use efficiency and nutritional quality in a diverse array of hydroponically cultivated microgreens. The findings indicate that light quality presents a dual challenge: while it stimulates growth in some crops, it may inhibit the growth of others. For instance, greens, such as lettuce and green basil, exhibited reduced biomass under the blue-enriched spectrum, whereas pigment-rich herbs and brassicas utilised the same light to enhance their growth. Water-use efficiency displayed a similar dichotomy, decreasing where stomata were widely open, yet increasing in species whose natural antioxidants facilitated safer light absorption, thereby preventing photoinhibition. Changes in chlorophyll content were limited to a few crops, while phenolic richness remained stable, except in isolated instances. Blue-leaning light reduced in some species the nitrate levels, which is significant for dietary guidelines. Instead, the results highlight the importance of adopting dynamic, crop-specific light spectra that evolve throughout the short microgreen lifecycle, combined with irrigation strategies tailored to each species' growth rate. This approach promises higher yields per litre of water and produces an improved phytonutrient profile, which is an appealing prospect for vertical farms seeking to minimise both their environmental footprint and nitrate levels. Future research should explore finer colour gradients, incorporate additional wavebands, and expand the testbed to include other specialty crops, thereby refining the guidelines for resource-efficient, nutrient-rich micro-produce cultivation.

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