

Interactive Effects of Spectrum and Intensity of RBW, FR and UV-A on Growth, Photosynthetic Performance, and Antioxidant Properties of Basil and Parsley Grown in Indoor Vertical Farm

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Article

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Abstract

Controlling the spectrum and intensity of artificial light offers an effective strategy to optimize crop productivity and nutritional quality in controlled-environment agriculture. This study evaluated the combined effects of red–blue–white light (RBW), supplemented with far-red (RBW + FR), and RBW supplemented with both FR and UV-A (RBW + FR+UVA) at three photosynthetic photon flux densities (PPFDs; 100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) on growth, photosynthetic traits, pigment composition, and antioxidant capacity of basil (*Ocimum basilicum* L.) and parsley (*Petroselinum crispum* L.). Across all light intensities, RBW + FR consistently enhanced vegetative growth in basil, increasing growth and biomass accumulation compared with RBW alone. In contrast, RBW + FR+UVA generally reduced growth but increased anthocyanin concentration, phenolic and flavonoid contents. Basil exhibited higher chlorophyll content, photosynthetic performance index (PI), and stomatal conductance under RBW + FR, particularly at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, indicating improved photosynthetic efficiency under low-light conditions. Parsley showed a weaker growth response to spectral treatments but demonstrated substantial increases in total phenolic content, flavonoids, and DPPH free-radical scavenging activity under RBW + FR+UVA, especially at reduced PPFDs. Overall, FR radiation primarily stimulated growth and photosynthetic performance, whereas UV-A acted as a biochemical elicitor, enhancing antioxidant capacity at the expense of biomass. These species-specific responses highlight the importance of tailored spectral strategies to balance yield and nutritional quality in low-energy indoor farming systems.

Introduction

The rapid expansion of controlled-environment agriculture (CEA), particularly multi-layer vertical farming systems, has intensified interest in optimizing light quality to improve crop productivity, energy-use efficiency, and nutritional value [1]. In CEA systems, artificial lighting accounts for one of the largest operational energy inputs; therefore, strategic spectral optimization is critical for maximizing physiological efficiency and crop quality under reduced photon flux density (PPFD). Light-emitting diode (LED) technology offers unprecedented flexibility in manipulating spectral composition, enabling precise delivery of specific wavebands such as red, blue, far-red, and ultraviolet-A radiation to regulate plant growth and metabolism [1, 2].

Red and blue light constitute the primary drivers of photosynthesis, directly influencing chlorophyll absorption, photosystem excitation, and carbon assimilation. Blue light additionally regulates stomatal conductance, chloroplast development, and photomorphogenic responses via cryptochrome and phototropin signaling pathways [3, 4]. Beyond photosynthetic efficiency, supplemental wavelengths, such as far-red and UV-A, function as regulatory signals that modulate plant architecture, resource allocation, and secondary metabolism without substantially increasing photosynthetic photon flux [5].

Far-red light plays a central role in shade-avoidance responses by mediating phytochrome perception of the red:far-red ratio, thereby influencing downstream hormonal signaling and gene expression. Supplemental far red has been shown to promote stem elongation, leaf expansion, canopy light interception, and biomass accumulation, particularly under low to moderate PPFD conditions [6, 7]. In CEA, far red can enhance photosynthetic performance indirectly by improving leaf morphology, reducing self-shading, and increasing the balance of photosystem excitation between PSI and PSII, thereby improving overall light-use efficiency [8, 9].

In contrast, UV-A radiation (315–400 nm) is widely recognized as an abiotic elicitor rather than a photosynthetic driver. UV-A activates cryptochrome- and UVR8-associated signaling pathways, inducing the biosynthesis of phenolic compounds, flavonoids, anthocyanins, and other antioxidant metabolites involved in photoprotection and oxidative stress mitigation [10, 11]. Under controlled exposure, UV-A has been reported to enhance nutritional quality and antioxidant capacity in leafy vegetables and herbs, often with minimal penalties to biomass production when applied

at low intensities or in combination with photosynthetically active radiation [12, 13]. However, the magnitude and direction of these responses are strongly species-dependent and influenced by background PPFD.

Despite increasing interest in far-red and UV-A supplementation, most existing studies have focused on low to moderate PPFDs (25 to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$), whereas information on their interactive effects under low PPFD conditions remains limited. This knowledge gap is particularly relevant for energy-efficient vertical farming systems, where crops are often cultivated at reduced light intensities to minimize electricity consumption. Under such conditions, plants may rely more heavily on spectral signaling rather than photon quantity to optimize growth, photosynthetic efficiency, and metabolic allocation [14, 15].

Basil (*Ocimum basilicum* L.) and parsley (*Petroselinum crispum* L.) are widely cultivated culinary herbs valued for their aromatic properties and health-promoting phytochemicals, including phenolics, flavonoids, and antioxidant compounds. Despite their commercial importance, these species differ substantially in morphology, photosynthetic capacity, pigment composition, and metabolic regulation. Basil is generally characterized by higher photosynthetic plasticity and secondary metabolite responsiveness, whereas parsley exhibits more conservative growth and pigment acclimation strategies [16, 17]. Such interspecific differences suggest that basil and parsley may respond distinctly to spectral modification, particularly under low PPFD regimes where resource allocation trade-offs between growth and defense metabolism become more pronounced. Understanding how FR and UV-A radiation interact with low light intensity to regulate growth, photosynthetic performance, and antioxidant capacity is therefore essential for developing crop- and species-specific lighting strategies in CEA systems. Optimized spectral combinations may enable simultaneous improvement of biomass production and nutritional quality while maintaining low energy inputs.

The objective of this study was to evaluate the effects of RBW, RBW + FR, and RBW + FR + UV-A lighting at 100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on (i) growth characteristics, (ii) photosynthetic pigment composition and chlorophyll fluorescence parameters, and (iii) phenolic content, flavonoids, anthocyanins, and antioxidant activity in basil and parsley. We hypothesized that supplemental far-red radiation would primarily enhance growth and photosynthetic performance whereas UV-A supplementation would preferentially stimulate secondary metabolite accumulation with distinct, species-specific responses under low PPFD conditions.

Result

Plant growth characteristics and photosynthetic activity

Basil growth was significantly affected by light intensity, spectral composition, and their interaction (Table 1). Plant height was greatest under RBW + FR at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and remained high under the same spectrum at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas RBW alone produced shorter plants at reduced intensities. Leaf number increased under far-red containing spectra at 100 and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared with RBW. Leaf area showed strong interaction effects, with maximum values under RBW + FR + UVA at 50 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while the lowest values occurred under RBW at low intensity. Root length was primarily influenced by light intensity, with longer roots observed at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ across spectra. Fresh and dry biomass were highest under RBW + FR at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and declined markedly at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, particularly under RBW.

Table 1
Growth characteristics of basil grown under different light intensities and spectral compositions

Growth characteristics/plant	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$			50 $\mu\text{mol m}^{-2} \text{s}^{-1}$			25 $\mu\text{mol m}^{-2} \text{s}^{-1}$		
	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA
Plant height (cm)	22 $\pm$ 3c	29 $\pm$ 4a	20 $\pm$ 4c	11 $\pm$ 3e	25 $\pm$ 4b	25 $\pm$ 5b	12.5 $\pm$ 3e	26.5 $\pm$ 5ab	21.5 $\pm$ 4c
Leaf No	22 $\pm$ 2b	26 $\pm$ 2a	19 $\pm$ 3c	11 $\pm$ 2e	22 $\pm$ 3b	25 $\pm$ 5a	10 $\pm$ 2e	14 $\pm$ 4d	12 $\pm$ 3de
Leaf area (cm ²)	14 $\pm$ 5d	24 $\pm$ 3b	20 $\pm$ 2c	11 $\pm$ 2e	17 $\pm$ 2c	26 $\pm$ 3a	3.4 $\pm$ 2f	10 $\pm$ 3e	25.7 $\pm$ 2a
Root length (cm)	15 $\pm$ 4c	17 $\pm$ 5c	14 $\pm$ 3c	20 $\pm$ 5a	19 $\pm$ 4ab	18 $\pm$ 5ab	14.1 $\pm$ 3c	16.7 $\pm$ 3bc	16.3 $\pm$ 3bc
Fresh weight (g)	5.7 $\pm$ 2c	8 $\pm$ 2a	5.8 $\pm$ 2c	1.0 $\pm$ 0e	4.9 $\pm$ 1d	6.8 $\pm$ 2b	1.7 $\pm$ 1e	3.7 $\pm$ 1d	4.7 $\pm$ 1cd
Dry weight (g)	0.5 $\pm$ 0c	0.7 $\pm$ 0a	0.4 $\pm$ 0d	0.05 $\pm$ 0f	0.35 $\pm$ 0e	0.48 $\pm$ 0c	0.7 $\pm$ 0f	0.3 $\pm$ 0e	0.4 $\pm$ 0d
Values are presented as means $\pm$ standard deviation (SD). Different letters within each row indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$. R (Red), B (blue), R (red), FR (far red), UVA (ultra violet A).									

Parsley growth was also modulated by light intensity, spectral composition, and their combined effects, with responses differing among traits (Table 2). Plant height showed pronounced treatment-dependent variation, with the greatest elongation observed under RBW + FR at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while the most restricted growth occurred under RBW + FR + UVA at the same intensity. In contrast, leaf number remained largely unchanged across all treatments, indicating limited sensitivity to either factor. Leaf area responded strongly to the interaction between intensity and spectrum, reaching its maximum under RBW + FR at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas notable suppression of leaf expansion was evident under RBW + FR + UVA, particularly under low-light conditions. Root development was influenced mainly by light intensity, with enhanced root length at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ across spectral treatments. Biomass accumulation was greatest under far-red-enriched spectra at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$. and at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but declined substantially at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 2

Growth characteristics of the parsley grown under different light intensities and spectral compositions

Growth characteristics/plant	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$			50 $\mu\text{mol m}^{-2} \text{s}^{-1}$			25 $\mu\text{mol m}^{-2} \text{s}^{-1}$		
	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA
Plant height (cm)	14.3 $\pm$ 3b	11.3 $\pm$ 2c	6.3 $\pm$ 2e	12.5 $\pm$ 3c	14.3 $\pm$ 3b	7.5 $\pm$ 2d	13 $\pm$ 3bc	15 $\pm$ 4a	5.5 $\pm$ 2e
Leaf no	4.5 $\pm$ 1a	5 $\pm$ 0a	5 $\pm$ 0a	4 $\pm$ 0a	4 $\pm$ 0a	4 $\pm$ 0a	4 $\pm$ 0a	4 $\pm$ 0a	4 $\pm$ 0a
Leaf area (cm ²)	10 $\pm$ 4c	9.7 $\pm$ 2c	8.7 $\pm$ 2cd	13.3 $\pm$ b3	26.0 $\pm$ a5	7.8 $\pm$ 2d	9 $\pm$ 3c	12 $\pm$ 3b	4 $\pm$ 2e
Root length (cm)	16.5 $\pm$ 3c	17.6 $\pm$ 4bc	20 $\pm$ 6ab	21 $\pm$ 6a	19 $\pm$ 5ab	20 $\pm$ 4ab	18 $\pm$ 4bc	17 $\pm$ 4bc	14 $\pm$ 5c
Fresh weight (g)	1.6 $\pm$ 0.1b	1.9 $\pm$ 0a	1.9 $\pm$ 0a	1.2 $\pm$ 0c	1.8 $\pm$ 0a	0.15 $\pm$ 0e	1.5 $\pm$ 0b	1.2 $\pm$ 0c	1.0 $\pm$ 0d
Dry weight (g)	0.21 $\pm$ 0ab	0.22 $\pm$ 0a	0.20 $\pm$ 0b	0.16 $\pm$ 0c	0.21 $\pm$ 0ab	0.75 $\pm$ 0a	0.15 $\pm$ 0c	0.10 $\pm$ 0d	0.12 $\pm$ 0d
Values are presented as means $\pm$ standard deviation (SD). Different letters within each row indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$. R (red), B (blue), R (red), FR (far red), UVA (ultraviolet A).									

The accumulation of total phenolics (TPC) and total flavonoids (TFC) was significantly affected by light intensity, spectral composition, and their combined effects in both basil and parsley, with distinct species-dependent responses (Table 3). In basil, far-red enrichment promoted phenolic accumulation, with peak TPC observed under RBW + FR at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas plants grown under RBW, particularly at low intensity, exhibited reduced phenolic levels. Flavonoid content in basil was also strongly regulated by spectral quality, with far red containing treatments resulting in higher TFC at 100 and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while a pronounced decline was evident at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ regardless of spectrum. In parsley, secondary metabolite concentrations were generally greater than in basil and showed a strong response to spectral enhancement. The highest TPC was recorded under RBW + FR + UVA at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas TFC reached its maximum under the same spectrum at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in parsley.

Table 3

Total phenolic content and total flavonoid content of basil and parsley grown under different light intensities and spectral compositions

Analysis (mg/g DW)	Plant species	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$			50 $\mu\text{mol m}^{-2} \text{s}^{-1}$			25 $\mu\text{mol m}^{-2} \text{s}^{-1}$		
		RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA
TPC	Basil	13.8 $\pm 4\text{d}$	18.2 $\pm 5\text{b}$	16.2 $\pm 4\text{c}$	16.5 $\pm 5\text{c}$	19.8 $\pm 4\text{a}$	14.8 $\pm 4\text{cd}$	12.5 $\pm 4\text{d}$	19.4 $\pm 5\text{ab}$	16.1 $\pm 4\text{c}$
	Parsley	16.9 $\pm 5\text{b}$	18.7 $\pm 3\text{ab}$	22 $\pm 5\text{a}$	12 $\pm 4\text{d}$	14 $\pm 5\text{c}$	14 $\pm 3\text{c}$	11.3 $\pm 3\text{d}$	11.2 $\pm 3\text{d}$	13.5 $\pm 2\text{c}$
TFC	Basil	6.5 $\pm 3\text{b}$	8.3 $\pm 2\text{a}$	4.3 $\pm 2\text{cd}$	4.5 $\pm 2\text{cd}$	8.9 $\pm 4\text{a}$	4.1 $\pm 2\text{d}$	3.4 $\pm 2\text{d}$	4.6 $\pm 2\text{c}$	3.8 $\pm 3\text{d}$
	Parsley	12.8 $\pm 3\text{c}$	18.4 $\pm 4\text{ab}$	18.9 $\pm 5\text{c}$	13.2 $\pm 3\text{c}$	16 $\pm 5\text{b}$	18.3 $\pm 5\text{ab}$	12.8 $\pm 4\text{c}$	10.5 $\pm 3\text{d}$	19 $\pm 5\text{a}$
Values are presented as means $\pm$ standard deviation (SD). Different letters within each row indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$. R (red), B (blue), R (red), FR (far red), UVA (ultraviolet A).										

Photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, and carotenoids, were significantly influenced by light intensity, spectral composition, and their interaction in both basil and parsley, with responses differing between species (Table 4). In basil, the highest chlorophyll *a* and *b* concentrations were observed under RBW + FR at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas plants grown under RBW exhibited consistently lower pigment levels across all light intensities. Carotenoid accumulation showed a comparable response, with far red-enriched spectra promoting greater concentrations at high irradiance. Reductions in pigment content were evident at 50 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, particularly under RBW + FR + UVA. In parsley, chlorophyll *a* and *b* displayed strong sensitivity to spectral quality, reaching maximum values under RBW + FR + UVA at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but declining sharply under the same spectrum at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Carotenoid levels in parsley were consistently enhanced by far-red supplementation across intensities, with the highest concentrations recorded under RBW + FR at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 4
Chlorophyll and carotenoid content of basil and parsley grown under different light intensities and spectral compositions.

Plant species	Analysis	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$			50 $\mu\text{mol m}^{-2} \text{s}^{-1}$			25 $\mu\text{mol m}^{-2} \text{s}^{-1}$		
		RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA
Basil	Chl <i>a</i>	397 $\pm$ 65d	681 $\pm$ 124a	615 $\pm$ 133ab	620 $\pm$ 127ab	372 $\pm$ 55d	429 $\pm$ 74c	459 $\pm$ 48c	503 $\pm$ 71bc	349 $\pm$ 57d
	Chal <i>b</i>	214 $\pm$ 71d	357 $\pm$ 112a	321 $\pm$ 88ab	306 $\pm$ 94b	175 $\pm$ 64e	191 $\pm$ 65de	213 $\pm$ 60d	239 $\pm$ 42c	167 $\pm$ 47e
	Car	101 $\pm$ 32c	168 $\pm$ 56a	154 $\pm$ 23ab	121 $\pm$ 36bc	97 $\pm$ 31c	87 $\pm$ 24d	102 $\pm$ 21c	115 $\pm$ 34bc	79 $\pm$ 26d
Parsley	Chl <i>a</i>	283 $\pm$ 77bc	240 $\pm$ 47c	363 $\pm$ 89a	318 $\pm$ 76ab	291 $\pm$ 84bc	94 $\pm$ 26e	296 $\pm$ 58bc	332 $\pm$ 66ab	265 $\pm$ 52c
	Chal <i>b</i>	127bc $\pm$ 42bc	113 $\pm$ 23c	187 $\pm$ 76a	168 $\pm$ 40ab	137 $\pm$ 46bc	12 $\pm$ 5e	106 $\pm$ 33c	135 $\pm$ 28bc	116 $\pm$ 45c
	Car	44 $\pm$ 16d	83 $\pm$ 21a	63 $\pm$ 20bc	45 $\pm$ 14d	78 $\pm$ 17ab	76 $\pm$ 11ab	33 $\pm$ 12e	77 $\pm$ 16ab	49 $\pm$ 16cd
Values are presented as means $\pm$ standard deviation (SD). Different letters within each row indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$. R (red), B (blue), R (red), FR (far red), UVA (ultraviolet A).										

Photosynthetic performance traits were significantly influenced by the treatment in both basil and parsley (Table 5). In basil, the maximum quantum efficiency of PSII (F_v/F_m) was highest under RBW at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (0.79) and RBW + FR at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (0.81), whereas pronounced reductions were observed under RBW + FR + UVA, particularly at low intensity (0.60 at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The performance index (PI) exhibited strong interaction responses, with a marked increase under RBW + FR at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (3.0) and substantially lower values under RBW + FR + UVA at high irradiance. Stomatal conductance in basil reached its maximum under RBW + FR + UVA at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (327 $\text{mmol m}^{-2} \text{s}^{-1}$). In parsley, F_v/F_m remained comparatively stable across treatments, peaking under RBW at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (0.78). Parsley PI was highest under RBW at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1.68) and RBW + FR + UVA at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1.64), while stomatal conductance decreased progressively with declining light intensity.

Table 5

Maximum quantum yield, and stomatal conductance of basil and parsley grown under different light intensities and spectral compositions. Values are presented as means $\pm$ standard deviation (SD). Different lowercase letters within each row indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$. R (red), B (blue), R (red), FR (far red), UVA (ultraviolet A).

Plant species	Analysis	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$			50 $\mu\text{mol m}^{-2} \text{s}^{-1}$			25 $\mu\text{mol m}^{-2} \text{s}^{-1}$		
		RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA	RBW	RBW + FR	RBW + FR+UVA
Basil	F_v/F_m	0.79 $\pm$ 0.2a	0.69 $\pm$ 0.2b	0.63 $\pm$ 0.1c	0.69 $\pm$ 0.2b	0.68 $\pm$ 0.2b	0.65 $\pm$ 0.2bc	0.73 $\pm$ 0.2ab	0.81 $\pm$ 0.2a	0.60 $\pm$ 0.2c
	PI	1.8 $\pm$ 0.4b	0.60 $\pm$ 0.1d	0.37 $\pm$ 0.1e	0.78 $\pm$ 0.2cd	0.41 $\pm$ 0.1e	0.71 $\pm$ 0.1cd	0.98 $\pm$ 0.2c	3.0 $\pm$ 0.8a	0.55 $\pm$ 0.2d
	gs	212 $\pm$ c	141 $\pm$ 34d	192 $\pm$ 33c	298 $\pm$ 76ab	288 $\pm$ 64ab	327 $\pm$ 77a	222 $\pm$ 69c	134 $\pm$ 24d	183 $\pm$ 38c
Parsley	F_v/F_m	0.69 $\pm$ 0bc	0.69 $\pm$ 0.3bc	0.65 $\pm$ 0.2c	0.68 $\pm$ 0.2bc	0.77 $\pm$ 0.2ab	0.75 $\pm$ 0.2ab	0.78 $\pm$ 0.2a	0.68 $\pm$ 0.2bc	0.77 $\pm$ 0.2ab
	PI	0.45 $\pm$ 0.2d	0.70 $\pm$ 0.2c	0.17 $\pm$ 0.1e	1.68 $\pm$ 0.3a	0.71 $\pm$ 0.2c	1.33 $\pm$ 0.4b	1.16 $\pm$ 0.4b	0.33 $\pm$ 0.1de	1.64 $\pm$ 0.1a
	gs	259 $\pm$ 76a	143 $\pm$ 46b	162 $\pm$ 37b	94 $\pm$ 13cd	143 $\pm$ 26b	118 $\pm$ 17bc	89 $\pm$ 11d	92 $\pm$ 22d	65 $\pm$ 17e

Anthocyanin accumulation was significantly affected by light intensity, spectral composition, and their interaction in basil (Fig. 1). Anthocyanin content increased from RBW to RBW + FR and was highest under RBW + FR + UVA at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, anthocyanin levels were lowest under RBW + FR but increased significantly with UVA supplementation (RBW + FR + UVA). At 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, FR- and UVA-enriched spectra resulted in significantly higher anthocyanin contents (RBW + FR and RBW + FR + UVA) compared with RBW alone.

Antioxidant capacity assessed by both DPPH radical scavenging activity (Fig. 2) and FRAP reducing power (Fig. 3) was significantly influenced by light spectrum and intensity. Across all treatments, parsley consistently exhibited higher antioxidant capacity than basil in both assays. At 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, basil showed moderate DPPH activity (75–83%), which increased slightly under RBW + FR, whereas parsley reached higher values (80–88%). Correspondingly, FRAP values ranged from 4,500–5,600 $\mu\text{M Fe}^{2+}$ in basil and 5,500–6,900 $\mu\text{M Fe}^{2+}$ in parsley, with higher reducing power under FR- and UVA-enriched spectra. At 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, basil DPPH activity declined under RBW + FR + UVA (60%), while parsley maintained high activity (88–92%); FRAP values peaked in parsley under RBW + FR + UVA (8,500 $\mu\text{M Fe}^{2+}$). At the lowest intensity (25 $\mu\text{mol m}^{-2} \text{s}^{-1}$), basil showed reduced DPPH activity under RBW (60%) and lower FRAP (3,700–4,200 $\mu\text{M Fe}^{2+}$), whereas FR and UVA supplementation partially mitigated this decline. In contrast, parsley exhibited the highest antioxidant capacity at low light, with DPPH values approaching 95–98% and FRAP values of 7,800–8,900 $\mu\text{M Fe}^{2+}$.

Total amino acid content is shown in Fig. 4. At 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, basil exhibited the highest amino acid concentration under RBW (30 mg g^{-1}), followed by RBW + FR (29 mg g^{-1}) and RBW + FR + UVA (27 mg g^{-1}). In contrast, parsley also showed its maximum amino acid content under RBW (31 mg g^{-1}), while values declined sharply under RBW + FR (8 mg g^{-1}) and were partially restored under RBW + FR + UVA (19 mg g^{-1}). At 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, amino acid content

decreased in both species; basil ranged from 27 mg g⁻¹ (RBW) to 13 mg g⁻¹ (RBW + FR), whereas parsley maintained relatively higher levels, peaking at 23 mg g⁻¹ under RBW + FR + UVA. At the lowest intensity (25 µmol m⁻² s⁻¹), amino acid accumulation was substantially reduced, particularly in basil (4–14 mg g⁻¹), while parsley consistently showed higher contents (9–14 mg g⁻¹). Overall, parsley accumulated more amino acids than basil under reduced light intensity, and UVA supplementation partially alleviated amino acid depletion in both species.

Figure 5 shows the total nitrate content of basil and parsley, and their interaction effect with light spectrum and intensities. At 100 µmol m⁻² s⁻¹, basil exhibited nitrate concentrations of 5 mg g⁻¹ under RBW, which decreased slightly under RBW + FR (4 mg g⁻¹) and remained similar under RBW + FR + UVA (4 mg g⁻¹). In contrast, parsley accumulated substantially higher nitrate at the same intensity, with values increasing from 6 mg g⁻¹ DW under RBW to 8 mg g⁻¹ under RBW + FR and reaching a maximum of 9 mg g⁻¹ under RBW + FR + UVA. At 50 µmol m⁻² s⁻¹, nitrate content declined in both species; basil maintained moderate levels (4 mg g⁻¹ DW under RBW and RBW + FR; 3 mg g⁻¹ DW under RBW + FR + UVA), whereas parsley showed markedly lower concentrations (2 mg g⁻¹ under RBW and RBW + FR; 1 mg g⁻¹ under RBW + FR + UVA). At the lowest intensity (25 µmol m⁻² s⁻¹), nitrate accumulation remained low in both species, with basil ranging from 3 to 4 mg g⁻¹ and parsley stabilizing at ~ 1 mg g⁻¹ across spectra. Overall, parsley accumulated higher nitrate than basil at high light intensity, whereas nitrate levels were strongly reduced at lower intensities, particularly under FR- and UVA-enriched spectra.

The PCA biplot reveals clear species-specific acclimation strategies to light spectrum and intensity (Fig. 6). Separation along PC1 indicates a dominant nitrogen–secondary metabolism axis, with positive loadings from amino acids, nitrate, TPC, and TFC. Parsley samples cluster toward positive PC1 values, demonstrating a greater propensity to allocate resources toward nitrogen assimilation and secondary metabolite synthesis, particularly under RBW + FR+UVA, consistent with UVA-driven elicitation of phenolic and flavonoid pathways.

In contrast, PC2 represents a photosynthetic pigment axis, driven by chlorophyll a, chlorophyll b, carotenoids, F_v/F_m , PI, and gs. Basil samples predominantly align along PC2, reflecting a strategy centered on optimizing photosynthetic efficiency and pigment accumulation, especially under RBW + FR, which enhanced PI and chlorophyll content. This suggests that far-red supplementation promotes efficient light harvesting and photochemical performance in basil.

The opposing orientation of nitrogen-related variables relative to pigment and photosynthetic traits highlights a carbon–nitrogen trade-off between the two species. While parsley prioritizes nitrogen storage and antioxidant defenses, basil preferentially channels assimilated carbon into photosynthetic machinery. Notably, RBW + FR+UVA shifts parsley toward higher PC1 and PC2 values (enhanced antioxidants with maintained photosynthetic function), whereas the same treatment drives basil downward along PC2 at lower PPFD, indicating photosynthetic stress under UVA when light intensity is limiting.

Discussion

Plant growth characteristics

The present study demonstrates that the growth responses of basil and parsley are jointly regulated by light intensity, spectral composition, and their interaction, with species-specific sensitivities (Tables 1 and 2). In basil, far-red supplementation markedly enhanced plant height, leaf number, and biomass, particularly at 100 µmol m⁻² s⁻¹ and 50 µmol m⁻² s⁻¹ light intensities. This response is consistent with phytochrome-mediated shade-avoidance signaling, where enrichment of far red light lowers the red:far-red ratio and shifts phytochrome to the inactive Pr form, promoting stem elongation, leaf expansion, and carbon allocation to shoot growth [8, 18]. The strong biomass

accumulation observed under RBW + FR at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ indicates that far red acted synergistically with sufficient photosynthetic photon flux to enhance whole-plant carbon gain, rather than merely inducing elongation.

Leaf area expansion in basil was maximized under combined FR + UVA, particularly at $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ intensities, suggesting an additive role of UVA in regulating leaf morphology. UVA is known to activate cryptochrome and UVR8 signaling pathways, which can stimulate epidermal cell expansion and leaf surface development while modulating auxin transport [19, 20]. Root length responses in both species were primarily driven by light intensity rather than spectral quality, with longer roots observed at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ intensity. This pattern reflects a shift in carbon partitioning toward belowground organs under non-saturating light, a strategy commonly observed in leafy vegetables grown under controlled environments [9]. Excessive far red did not further stimulate root growth, supporting the view that far red signaling preferentially enhances shoot competitiveness rather than root development.

Parsley exhibited comparatively weaker shoot elongation responses to spectral manipulation, indicating reduced sensitivity to phytochrome-mediated shade signals relative to basil. However, parsley leaf area and biomass responded strongly to far red enrichment at moderate intensity, highlighting a trait-dependent rather than uniform species response. The stability of leaf number across treatments suggests that parsley leaf initiation is more genetically constrained and less responsive to light quality, consistent with prior reports for Apiaceae crops [21]. At $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, growth suppression in both species particularly under RBW reflects insufficient photosynthetic energy to sustain biomass accumulation. The partial mitigation of this decline by far red and UVA supports recent evidence that spectral optimization can improve light-use efficiency under suboptimal irradiance, although it cannot fully substitute for photon quantity [8, 13].

Total phenolics and flavonoids Content

In basil, far-red light significantly enhanced total phenolic content (TPC) at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$. This response is consistent with phytochrome-mediated regulation of the phenylpropanoid pathway, where reduced red:far-red ratios shift phytochrome toward the inactive Pr form, stimulating phenylalanine ammonia-lyase (PAL) activity and downstream phenolic biosynthesis [8, 18]. However, at $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, basil TPC and TFC declined across spectra, indicating that carbon limitation outweighed spectral signaling, restricting secondary metabolite synthesis.

Parsley consistently exhibited higher TPC and TFC than basil, with maximal values under RBW + FR + UVA, indicating greater biochemical sensitivity to spectral enrichment. UVA radiation is known to activate UVR8 and cryptochrome signaling, which upregulates transcription factors such as HY5 that directly induce flavonoid biosynthetic genes, enhancing antioxidant compound accumulation even under suboptimal light conditions [20, 22]. The highest TFC under RBW + FR + UVA at $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ suggests that UVA can partially compensate for reduced photon flux by promoting stress-protective secondary metabolism in parsley.

Photosynthetic Pigment Responses and Antioxidant Capacity

The significant changes in chlorophyll *a*, chlorophyll *b*, and carotenoids in basil under far-red-containing spectra at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ indicate improved photosynthetic antenna development and increased light-harvesting capacity (Table 4). Far red light has been shown to increase chlorophyll accumulation by promoting leaf expansion and reallocating nitrogen toward photosynthetic pigments [9]. In contrast, pigment reductions observed under RBW + FR + UVA at $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ suggest that UVA may impose mild oxidative stress leading to pigment downregulation as an acclimation response.

Parsley showed a distinct pigment strategy, with maximal chlorophyll accumulation under RBW + FR + UVA at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The strong carotenoid response to far red light across intensities supports their role in non-photochemical quenching and ROS scavenging, particularly in species adapted to variable light environments [23].

The progressive increase in anthocyanin content from RBW and RBW + FR + UVA at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ suggests that far-red and UVA acted synergistically to stimulate anthocyanin biosynthesis when sufficient photosynthetic energy was available. Far-red enrichment shifts phytochrome photoequilibrium, promoting assimilate allocation and transcriptional activation of phenylpropanoid pathway genes, whereas UVA activates UVR8–HY5 signaling, directly upregulating chalcone synthase (CHS) and dihydroflavonol reductase (DFR), key enzymes in anthocyanin biosynthesis [18, 20]. At 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the reduction of anthocyanin content under RBW + FR indicates that far red alone favored growth-related carbon use over secondary metabolism. However, the strong recovery under RBW + FR + UVA highlights the role of UVA as a stress-signaling wavelength, capable of redirecting carbon flux toward protective pigments even under moderate light limitation. At the 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ intensity, the consistently higher anthocyanin levels under far-red and UVA-enriched spectra suggest that anthocyanins function primarily as photoprotective and antioxidant compounds, compensating for reduced photosynthetic capacity and increased excitation pressure [22].

The antioxidant responses measured by DPPH and FRAP closely paralleled anthocyanin trends, confirming that enhanced antioxidant capacity was largely driven by phenylpropanoid-derived compounds. Parsley consistently exhibited higher DPPH scavenging activity and FRAP reducing power than basil, indicating a greater intrinsic capacity for secondary metabolite accumulation. This species-specific response likely reflects higher baseline expression of flavonoid biosynthetic genes and a stronger UVA sensitivity in parsley, as reported for Apiaceae crops [21]. The pronounced increase in parsley antioxidant capacity under RBW + FR + UVA at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light further supports the hypothesis that UVA preferentially enhances defensive metabolism rather than growth, especially under carbon-limited conditions.

Photosynthetic efficiency and gas exchange

Maximum quantum yield (F_v/F_m) remained relatively stable across treatments, indicating that none of the light regimes caused severe photoinhibition (Table 5). However, reductions of F_v/F_m under RBW + FR + UVA at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in basil suggest increased excitation pressure and oxidative stress, consistent with reduced pigment levels and lower PI. The strong increase in PI under RBW + FR at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ reflects enhanced electron transport efficiency and energy utilization, likely driven by improved source–sink balance under far red enrichment [8]. Stomatal conductance (g_s) responses further highlight species differences. Basil exhibited maximal g_s under FR + UVA at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, suggesting improved stomatal opening, whereas parsley showed a progressive decline in g_s with decreasing light intensity, reflecting tighter stomatal regulation.

Amino acid and nitrate accumulation

Total amino acid and nitrate contents were strongly influenced by the treatment (Fig. 4 and Fig. 5). In both species the highest amino acid concentrations were observed under RBW at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, indicating that adequate photosynthetic photon flux favored carbon skeleton supply (e.g., 2-oxoglutarate) required for amino acid biosynthesis through the GS–GOGAT pathway. The gradual reduction in amino acid content under far red and UVA-enriched spectra at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ suggests a partial reallocation of assimilated carbon toward growth and secondary metabolism rather than nitrogen assimilation [23, 24].

Parsley exhibited a sharper decline in amino acid content under RBW + FR than basil, particularly at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, indicating stronger sensitivity of nitrogen metabolism to far red induced changes in source–sink relations. Far-red light is known to enhance shoot elongation and leaf expansion, increasing carbon demand and potentially limiting nitrogen assimilation when nitrate reduction does not scale proportionally (Carotti et al., 2024). The partial recovery of amino acid levels under RBW + FR + UVA suggests that UVA may stimulate stress-responsive nitrogen metabolism, possibly by enhancing amino acid synthesis as osmoprotectants and antioxidants under altered light conditions [22].

The pronounced decline in amino acid content especially in basil reflects carbon limitation, where reduced photosynthesis constrains the supply of carbon backbones for amino acid biosynthesis at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Parsley consistently maintained higher amino acid levels than basil under reduced irradiance, indicating a more conservative metabolic strategy and greater nitrogen-use efficiency under light limitation.

Higher nitrate levels in parsley at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, particularly under far red and UVA-enriched spectra, indicate a decoupling between nitrate uptake and nitrate reduction. This suggests that nitrate reductase activity did not fully match nitrate uptake under these spectral conditions, leading to nitrate accumulation [25, 26]. In contrast, the strong reduction in nitrate content at 50 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in both species especially under far red and UVA indicates enhanced nitrate assimilation relative to uptake or reduced nitrate transport, a response commonly associated with lower growth rates and altered redox balance under low light.

PCA-based integration of physiological responses

The PCA highlights contrasting acclimation strategies of basil and parsley to light spectrum and intensity, reflecting differential regulation of carbon allocation, nitrogen and secondary metabolism. Parsley clustered along PC1, driven by amino acids, nitrate, TPC, and TFC, indicating preferential investment in nitrogen assimilation and phenylpropanoid biosynthesis, particularly under RBW + FR + UVA. This response is consistent with UVA-induced UVR8–HY5 signaling, which transcriptionally activates flavonoid and phenolic biosynthetic pathways and promotes antioxidant metabolism [20, 22]. The concurrent association with nitrate suggests partial uncoupling of nitrate uptake from growth, allowing nitrogen retention and metabolite accumulation.

In contrast, basil aligned primarily with PC2, dominated by chlorophylls, carotenoids, F_v/F_m , PI, and g_s , reflecting a strategy focused on photosynthetic optimization and efficient light harvesting. Far-red supplementation under RBW + FR likely altered phytochrome photoequilibrium, enhancing chlorophyll accumulation and photochemical efficiency [7, 8]. Together, the PCA reveals a growth–metabolism trade-off, with FR favoring photosynthetic performance in basil and UVA reinforcing antioxidant and nitrogen-related metabolism in parsley, emphasizing the need for species-specific spectral optimization.

Overall, the PCA confirms that RBW + FR provides the most balanced response across species, whereas UVA acts as a metabolic elicitor in parsley but imposes photochemical constraints in basil, particularly at reduced PPFD. These multivariate patterns underscore the importance of tailoring spectral strategies to species-specific physiological priorities in controlled-environment agriculture.

Conclusion

This study demonstrates that light intensity and spectral composition interactively regulate growth, photosynthetic performance, nitrogen metabolism, and phytochemical accumulation in basil and parsley, with pronounced species-specific responses. Far-red supplementation primarily enhanced photosynthetic efficiency, pigment accumulation, and

biomass production in basil, reflecting a strategy focused on optimizing light harvesting and carbon gain. In contrast, the inclusion of UVA particularly in combination with far-red—shifted resource allocation toward nitrogen retention and secondary metabolite synthesis in parsley, thereby increasing antioxidant capacity and phenylpropanoid accumulation. Multivariate PCA analysis confirmed these contrasting acclimation strategies, highlighting a trade-off between photosynthetic optimization and metabolic investment driven by spectral cues. Overall, these findings emphasize that targeted spectral management, rather than uniform lighting strategies, is essential to simultaneously optimize productivity and nutritional quality in controlled-environment agriculture.

Materials and Methods

Plant materials and growing conditions

Basil (*Ocimum basilicum* L. cv. MIA Prosper active DMR) and parsley (*Petroselinum crispum* L cv. Katinka) seeds (Johnny seeds.com, USA) were germinated on moistened rockwool cubes (1.2-inch rockwool cubes). After emergence (after three weeks of sowing), uniform seedlings were selected and transferred to a deep-flow hydroponic system (10 L capacity plastic container growing pot) providing commercial nutrient solution (Humboldt's Secret Supplies, California, USA). The nutrient solution was applied at 6 ml/gallon of water, according to the manufacturer's dosage guidelines. Both plant species were grown in an indoor plant factory at $23 \pm 2^\circ\text{C}$, 35–40% relative humidity, and a 16 h light/8 h dark photoperiod. The hydroponic nutrient solution was maintained at an EC of $1.5\text{--}2.0\text{ dS m}^{-1}$ and pH 6.0 to 6.5, adjusted as needed with 1 N HCl. The solution was aerated continuously and replaced every 2 weeks to prevent nutrient/salt deposition or microbial growth. This experiment was repeated twice; each study collected data from 6 plants per treatment (total plants studied: $6 \times 2 = 12/\text{treatment}$), and plants were grown for 30 days.

Artificial LED light treatments

Five artificial LED light spectra, such as red (R) 660 nm, blue (B) 430 nm, far red (FR) 730 nm, UVA, 320 nm and white (W) warm white (correlated color temperature CCT 2700K) were used (LED panel grow light.com, China). Three spectral combinations were used based on the ratio of spectral intensities of total photosynthetic photon flux density (PPFD) as follows: RBW = 1:1:3; RBW: FR = 1:1:3:0.5; and RBW: FR: UVA = 1:1:3:0.5:0.5. Each light combination had three different light intensities 100 ± 10 ; 50 ± 5 and $25 \pm 5\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$. Total PPFD was measured at the canopy level using a calibrated quantum sensor (LI-250A, LI-COR Biosciences, USA). Adjustments were made by modifying panel height to compensate for plant height changes during growth across all treatments.

Analysis of maximum quantum yield, stomatal conductance

Non-destructive photosynthetic data were collected on 30 days post-transplantation of basil and parsley. The maximum quantum yield (F_v/F_m) was measured using a chlorophyll fluorometer after 30 min dark adaptation, along with Photosynthetic Index (PI) using a portable chlorophyll fluorimeter (Hansatech Pocket PEA Chlorophyll Fluorimeter). The non-destructive stomatal conductance was measured using SC-1 Leaf Porometer. In each treatment, six plants per treatment were used for data collection.

Plant morphological measurements

After 30 days of transplanting, six plants from each treatment/study (a total of 12 plants) were harvested for morphological and biochemical analysis. The following parameters were recorded including plant height (cm): measured from the base to the apex using ruler; leaf number: total fully expanded leaves per plant; leaf area (cm^2):

measured using a leaf area meter (LI-3100, LI-COR, USA); root length (cm): measured using ruler; fresh weight (FW) and dry weight (DW) using electric balance. Dry weight was determined after oven-drying samples at 65°C for 72 h.

Chemical analysis

Sample preparation

For the analysis of polyphenol compounds, the first set consisted of six plants from replication 1, and the second set consisted of six plants from replication 2. Each set of plants was dried and ground into a fine powder, then kept in an airtight Ziplock bag. During analysis, each set was triplicated; therefore, a total of 6 samples from 2 sets were analyzed. In the final calculation, six samples were averaged and presented as means $\pm$ SD.

Briefly, 0.5 g of dried sample was mixed with 30 mL of 80% methanol in a 50 mL Falcon plastic tube. The mixture was shaken at 300 rpm overnight (12 to 16 hr) using an electric shaker. The extract was then filtered through Whatman No. 1 filter paper (0.20 mm, Whatman, 5B Cytiva, Marlborough, MA, USA), and the extracted eluent was stored at 4°C in a refrigerator until further analysis.

Estimation of Total Phenolic Content

The total phenolic content (TPC) of basil and parsley was quantified according to Folin–Ciocalteu method [27]. Briefly, 1 mL of the extract was mixed with 0.2 mL of 1 N Folin–Ciocalteu reagent in a test tube. The mixture was then increased by adding 1.8 mL of deionized water, vortexed, and allowed to react for 3 min. Subsequently, 0.4 mL of 10% (v/v) Na_2CO_3 solution was added, and the final volume was adjusted to 4 mL with 0.6 mL of deionized water. After incubation for 1 h at room temperature, the absorbance was measured at 725 nm using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). The TPC was calculated from a gallic acid calibration curve and expressed as mg CE g⁻¹ dry weight, DW.

Determination of Total Flavonoid Content

The total flavonoid content (TPC) was determined following the method of Ghimeray et al. [28] with minor modifications. Briefly, 0.5 mL of sample extract was mixed with 0.1 mL of 10% aluminum nitrate and 0.1 mL of 1 M potassium acetate solution. The mixture was then diluted to a final volume of 4 mL with 3.3 mL of 80% methanol, vortexed, and incubated for 40 minutes. Absorbance was recorded at 415 nm using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). The TF content was expressed as milligrams of coumarin equivalents per gram of dry weight (mg CE g⁻¹ dry weight, DW).

Chlorophyll (Chl) and Carotenoid (Car) Determination

For the determination of photosynthetic pigments, the fresh leaves (50 mg) from each treatment (three plants/treatment, a total of six samples made from two replications) were extracted (10 mL of 80% acetone) and placed at room temperature for 30 min. The collected extract was transferred into a tube and centrifuged at 4000 rpm for 10 min. The absorbance was taken at 647, 663 and 470 nm, respectively using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). Chlorophyll *a*, Chlorophyll *b*, and Carotenoid were determined according to the formula proposed by Lichtenthaler [29] and expressed as mg g⁻¹ fresh weight, FW:

$$\text{Chl } a = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$\text{Chl } b = 21.50 \times A_{647} - 5.10 \times A_{663}$$

$$\text{Car} = (1000 \times A_{470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b) / 198$$

Anthocyanin Determination

Total anthocyanins were analyzed according to the modified protocol described by Revilla et al. [30]. Each fresh leaf sample (30 mg) (sample preparation same as pigment analysis) was dissolved in 5 mL of 2% HCl in methanol for 36 h at 4°C, then centrifuged ($1446 \times g$ for 15 min), and the supernatant was separated and used as a stock solution for anthocyanins analysis. Following that, 400 μ L of the collected supernatant (each sample) was diluted with 2 mL potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.025 M, pH 1.0). (0.4 M, pH 4.5). After 15 min, each solution was filtered, and the absorbance was measured at 515 nm; maximum absorbance was confirmed in separate scans using a UV-5000 VIS-NIR spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). Total anthocyanins were calculated as the equivalent value of cyaniding-3-glucoside and expressed as $\text{mg } 100 \text{ g}^{-1} \text{ FW}$.

Amino acid analysis

Total amino acid content was determined using the ninhydrin–citrate buffer method [31]. A citrate buffer (pH 5.5) was prepared from citric acid monohydrate and trisodium citrate dihydrate, and a fresh ninhydrin reagent was made by dissolving 2 g of ninhydrin in 100 mL of DMSO and diluting to volume with the citrate buffer. For the assay, 1.0 mL of each sample solution (sample preparation same as TPC) was transferred to a clean test tube, followed by the addition of 1.0 mL of ninhydrin reagent. The tubes were mixed thoroughly and heated in a water bath (at 80°C) for 10 minutes to allow color development. After heating, the tubes were cooled to room temperature for 5 minutes, and 2.0 mL of citrate buffer was added to each tube, bringing the total volume to 4.0 mL. The absorbance of the resulting purple solution was measured at 570 nm against a reagent blank within 30 minutes using a UV–Vis spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). A standard calibration curve was prepared by plotting absorbance against amino acid concentration, and the amino acid content was expressed as $\text{mg g}^{-1} \text{ DW}$.

Total Nitrate content analysis

Nitrate concentration in the plant extracts was determined using the salicylic acid method as described by Cataldo et al. [32] with minor modifications. Briefly, 0.5g of finely ground dried plant samples were extracted with 10 mL of deionized water by shaking for 12 h at room temperature using an electric shaker (300 rpm). The extract was then filtered through Whatman No. 1 filter paper (0.20 μ m, Whatman, 5B Cytiva, Marlborough, MA, USA). For the assay, 0.2 mL of the clear extract was mixed with 0.8 mL of 5% (w/v) salicylic acid prepared in concentrated sulfuric acid (H_2SO_4). The mixture was allowed to stand at room temperature for 20 min to allow color development. Subsequently, 19 mL of 2 N sodium hydroxide (NaOH) was carefully added to neutralize and complete the reaction. After cooling to room temperature, the absorbance was measured at 410 nm using a UV-spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). A standard calibration curve was prepared using potassium nitrate (KNO_3) solutions ranging from 0 to 100 $\mu\text{g mL}^{-1}$. Nitrate concentration in the samples was calculated from the standard curve and expressed as milligrams of nitrate per gram of dry weight ($\text{mg NO}_3^- \text{ g}^{-1} \text{ dw}$).

DPPH Free Radical Scavenging Capacity

The antioxidant capacity was determined based on the scavenging activity of the stable 2,2-diphenyl-1 picryl hydrazyl (DPPH) free radical according to methods described by Braca et al. [33]. The DPPH solution was prepared (5.914 mg of DPPH powder dissolved in 100 mL of 100% methanol) to maintain an absorbance range of 1.1–1.3 by a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). Briefly, 1 mL of stock solution was added to 3 mL of DPPH solution. The blank sample was prepared by adding 1 mL of methanol instead of the stock extract to 3 mL of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature in the dark for 30

min. The absorbance was measured at 517 nm using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). The percentage inhibition activities of the plant samples were calculated against a blank sample using the following equation:

$$\text{Inhibition (\%)} = [(\text{blank sample} - \text{extract sample}) / \text{blank sample}] \times 100.$$

2.5.7. Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power of the samples was estimated according to the FRAP assay as described by Pulido [34]. In brief, 1 mL of extracted plant sample was mixed with 1 mL of 0.2 M phosphate buffer, maintaining a pH of 6.6. The mixture was then incubated at 50°C for 20 min. After incubation, 1 mL of trichloroacetic acid (TCA) was added to the solution, which was then centrifuged at 3000 rpm for 10 min. The collected supernatant was diluted with distilled water at a 1:1 ratio. Finally, 0.25 mL of 0.1% ferric chloride was added, and the absorbance was measured at 700 nm by a spectrophotometer UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan).

Statistical analysis

For polyphenol analysis, plant material was collected from two independent biological replications, each consisting of six plants. Each biological replication was analyzed in triplicate (technical replicates). Statistical analyses were performed using IBM SPSS Statistics (version 31.0.1.0; IBM Corp., Armonk, NY, USA). Data were analyzed using a two-way analysis of variance (ANOVA) with light intensity (100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and light spectrum (RBW, RBW + FR, and RBW + FR + UVA) as fixed factors. The main effects of light intensity, light spectrum, and interaction were evaluated for all growth parameters and phenolic compounds. The significant differences were detected at $P < 0.05$, and mean separation was performed using Tukey's honestly significant difference (HSD) test. Results are presented as the mean $\pm$ SD (standard deviation).

Declarations

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Author Contribution

MTN and MOKA conceptualize the experiment. MOKA conducted the research, analyzed the data, and drafted the manuscript. MTN acquired the project, oversaw the project progress, and revised and approved the final manuscript.

Data Availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

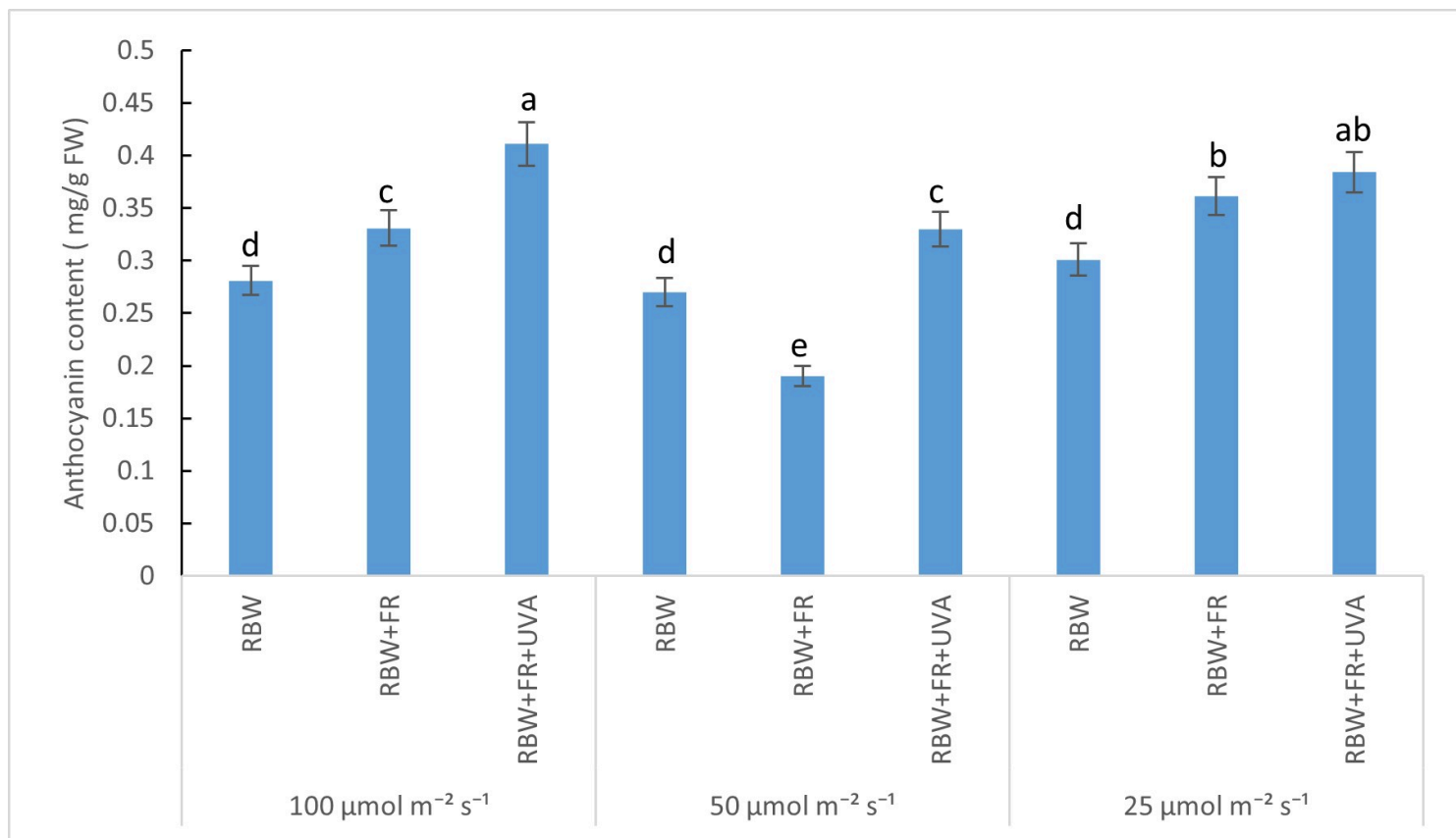


Figure 1

Effects of light intensity and spectral composition on anthocyanin content of basil. Anthocyanin content ($\text{mg g}^{-1} \text{FW}$) under three light intensities (100, 50, and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$) and three spectral treatments (RBW, RBW + FR, and RBW + FR + UVA). Bars represent, means $\pm$ SD. Different letters indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

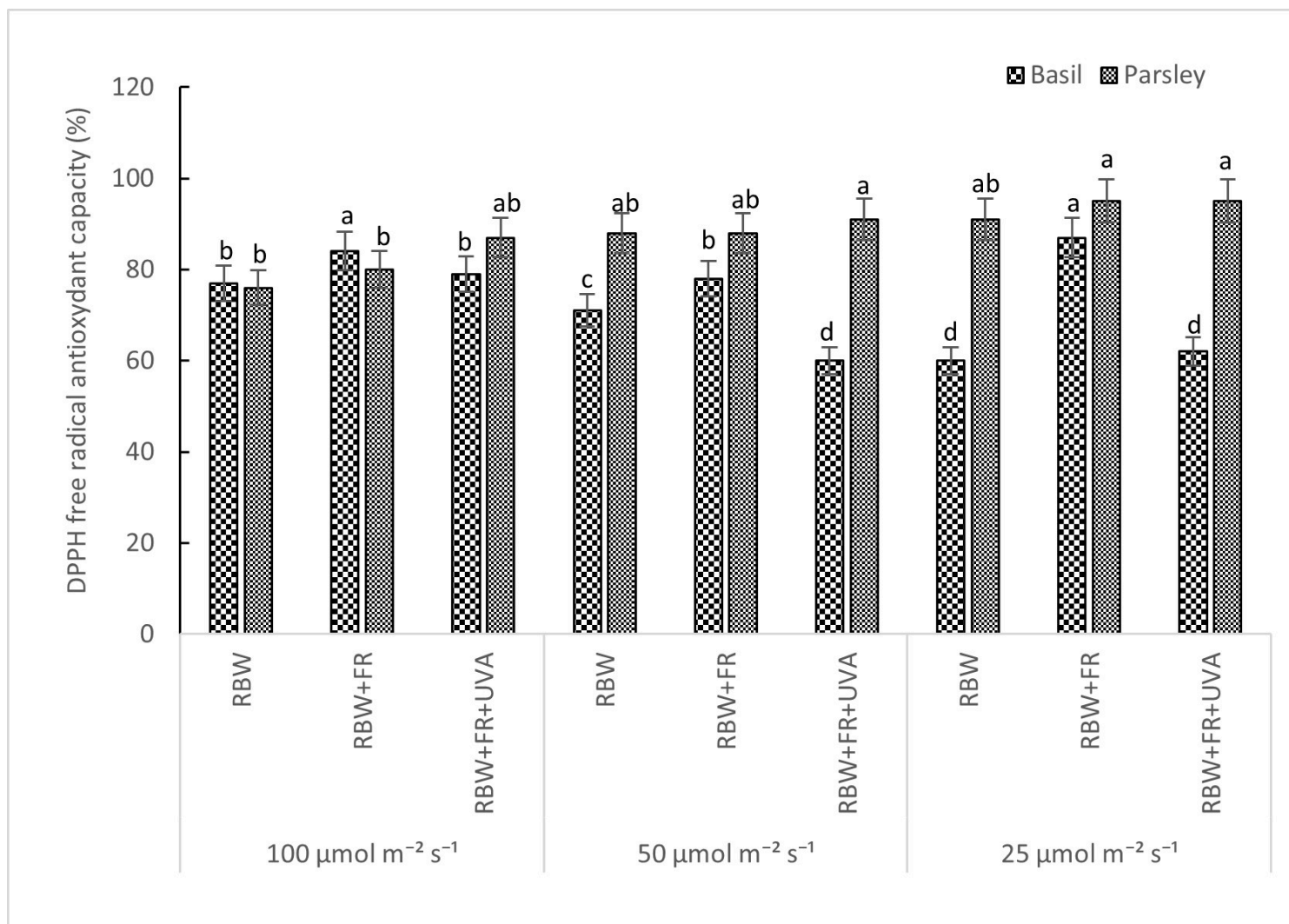


Figure 2

Effects of light intensity and spectral composition on DPPH free radical scavenging capacity of basil and parsley. DPPH antioxidant capacity (%) under three light intensities (100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and three spectral treatments (RBW, RBW + FR, and RBW + FR + UVA). Bars show means $\pm$ SD. Basil and parsley are shown separately for each treatment. Different letters indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

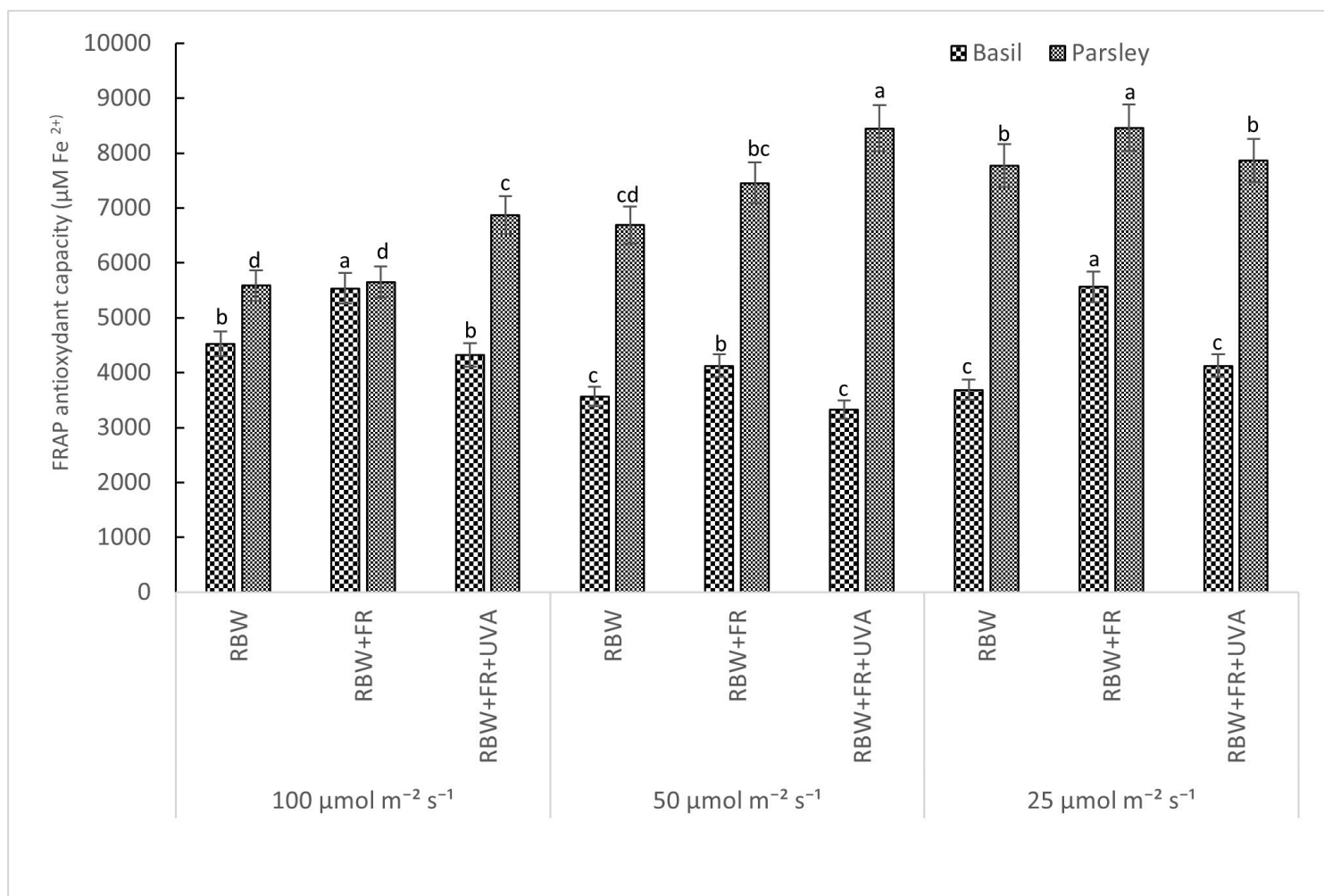


Figure 3

Effects of light intensity and spectral composition on DPPH free radical scavenging capacity of basil and parsley. DPPH antioxidant capacity (%) under three light intensities (100, 50, and 25 μmol m⁻² s⁻¹) and three spectral treatments (RBW, RBW + FR, and RBW + FR + UVA). Bars show means ± SD. Basil and parsley are shown separately for each treatment. Different letters indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at P < 0.05.

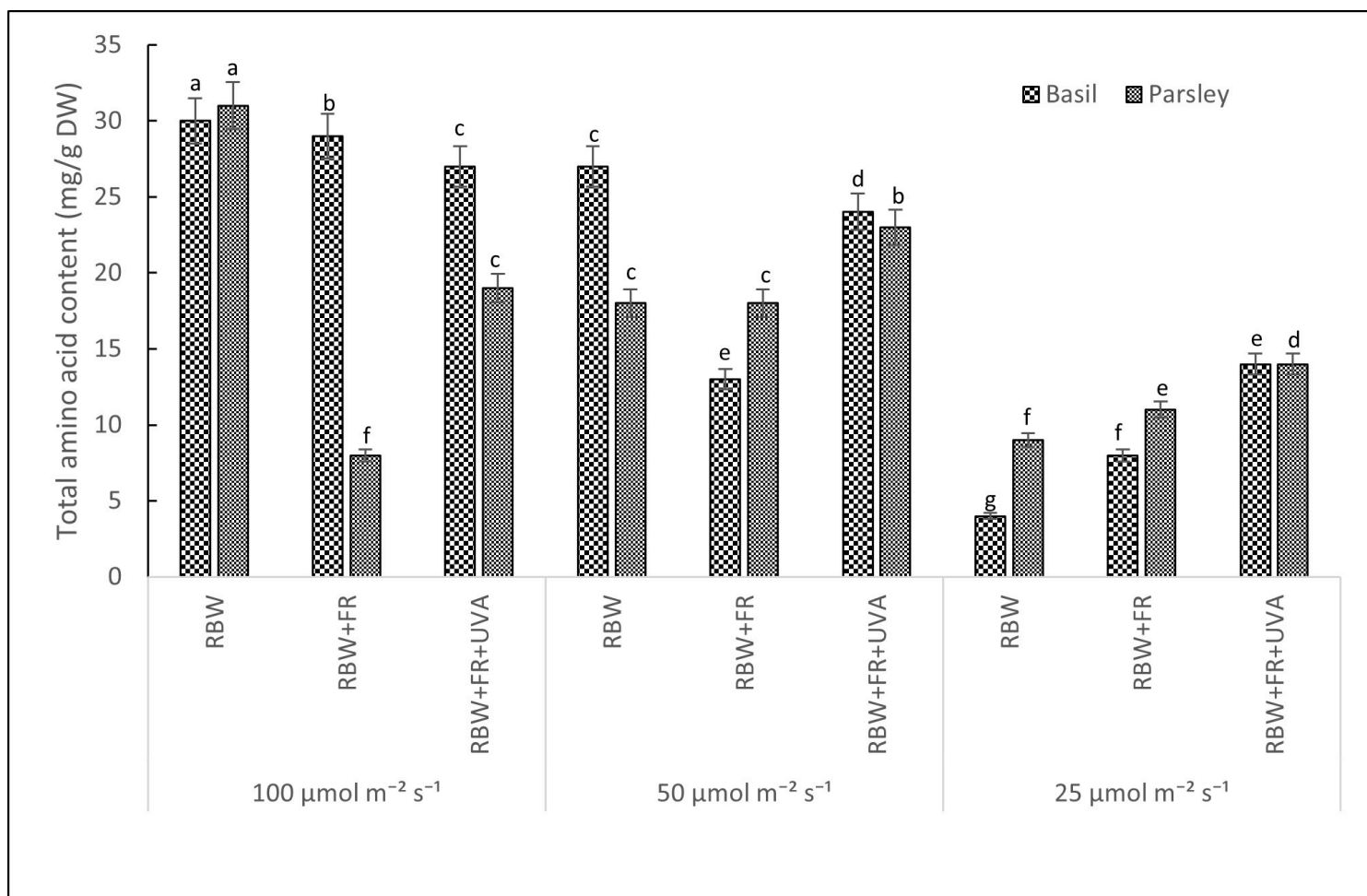


Figure 4

Effects of light intensity and spectral composition on total amino acid content of basil and parsley. DPPH antioxidant capacity (%) under three light intensities (100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and three spectral treatments (RBW, RBW + FR, and RBW + FR + UVA). Bars show means $\pm$ SD. Basil and parsley are shown separately for each treatment. Different letters (if applicable) indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

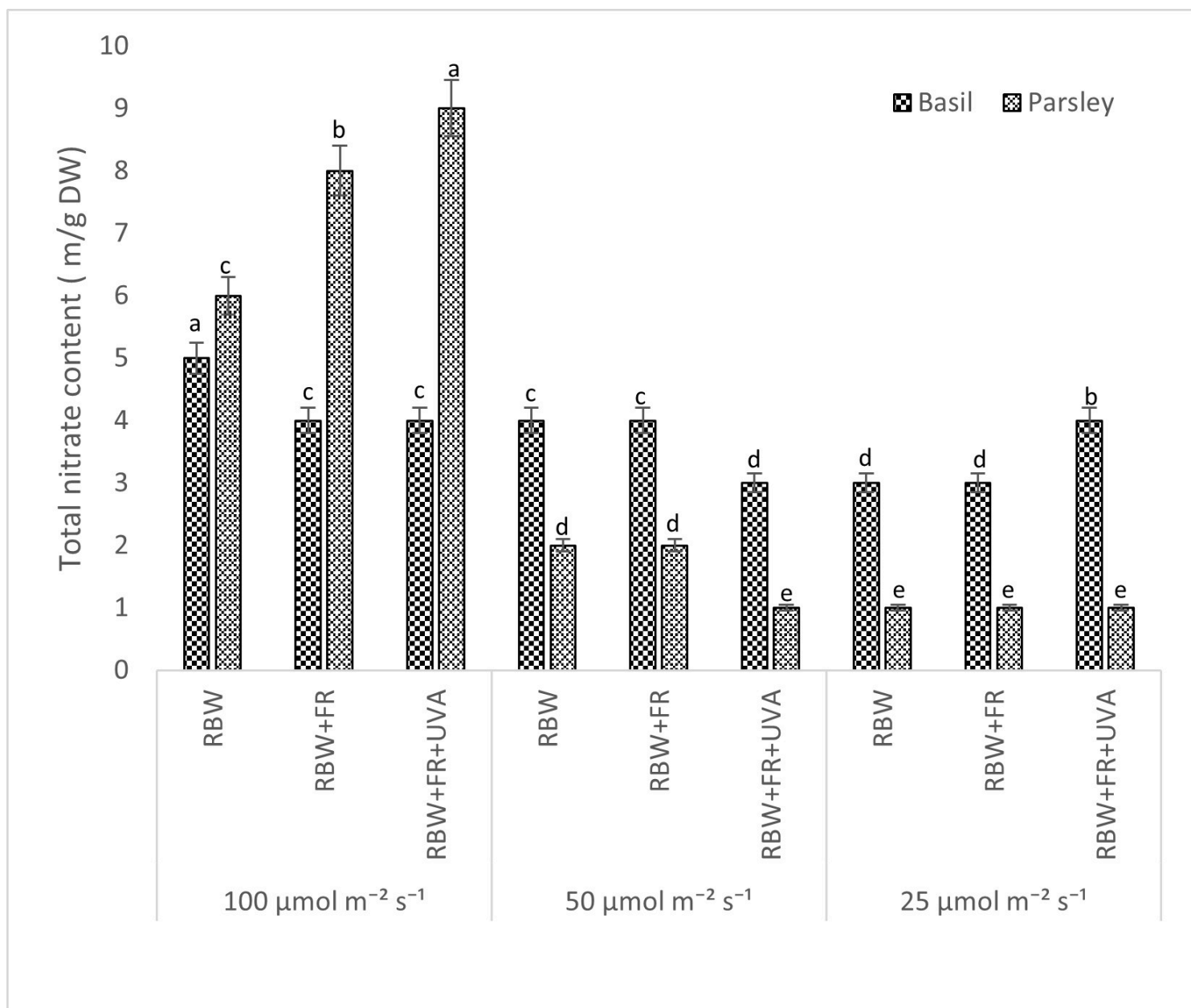


Figure 5

Effects of light intensity and spectral composition on total nitrate content of basil and parsley. DPPH antioxidant capacity (%) under three light intensities (100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and three spectral treatments (RBW, RBW + FR, and RBW + FR + UVA). Bars show means $\pm$ SD. Basil and parsley are shown separately for each treatment. Different letters (if applicable) indicate significant differences among treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

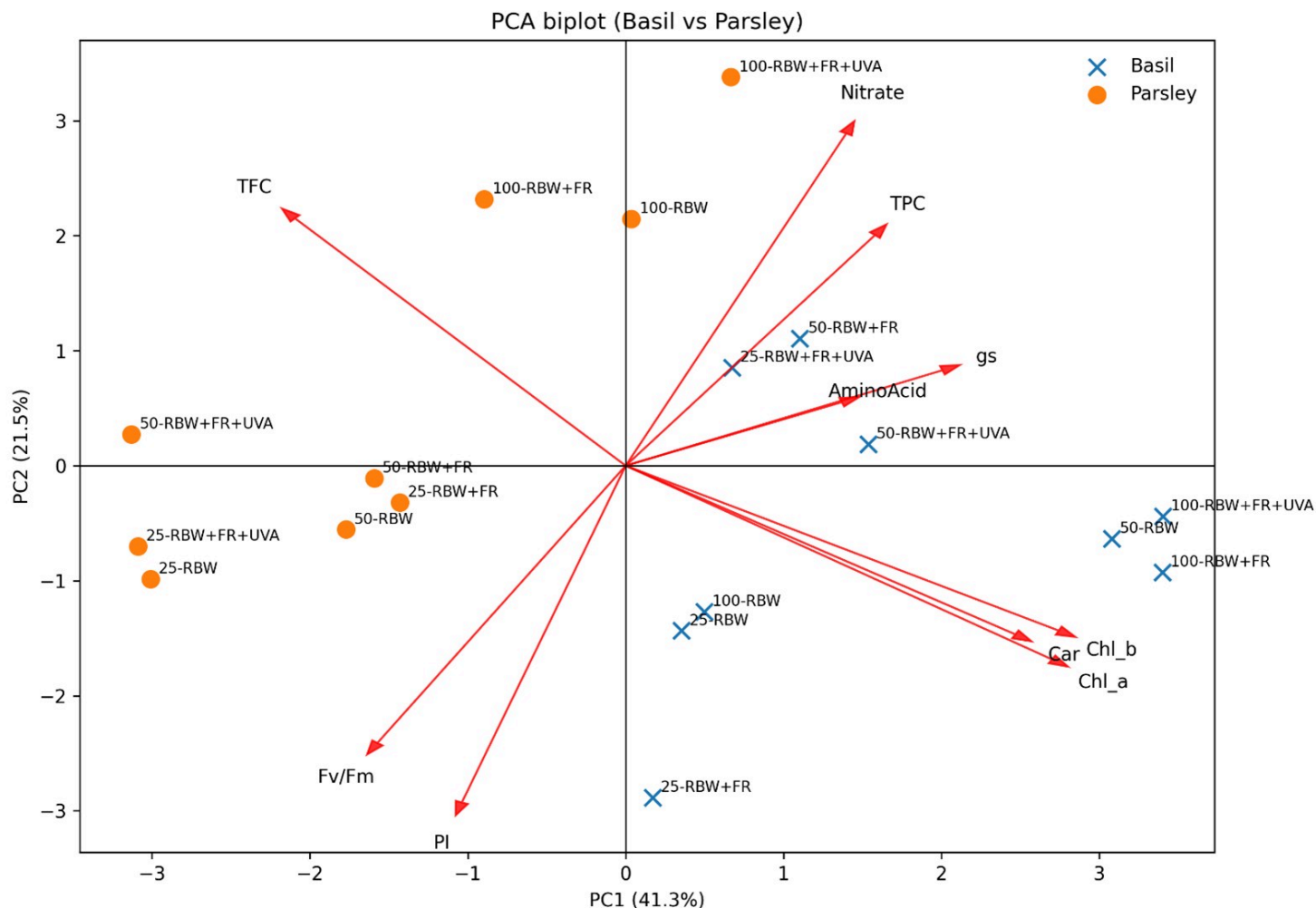


Figure 6

Principal component analysis (PCA) biplot illustrating the multivariate relationships among photosynthetic traits, pigment composition, nitrogen metabolites, and antioxidant properties in basil and parsley grown under different light spectra and PPFD levels. The PCA biplot illustrates the distribution of basil (x) and parsley (●) grown under RBW, RBW+FR, and RBW+FR+UVA at 100, 50, and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$. PC1 reflects nitrogen metabolism and secondary metabolites (amino acids, nitrate, TPC, TFC), while PC2 represents photosynthetic performance and pigment traits (chlorophylls, carotenoids, Fv/Fm, PI, gs). Red vectors indicate variable loadings, and sample proximity to vectors denotes trait associations. Species separation indicates distinct acclimation strategies to light spectrum and intensity.