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Optimizing Light Use Efficiency and Quality of Indoor Organically Grown Leafy Greens by Using Different Lighting Strategies

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Abstract: Vertical farming is experiencing significant growth, and the optimization of artificial lighting is essential for enhancing the sustainability of this growing system. Therefore, the aim of this study was to examine how light segmentation, the incorporation of a low-intensity lighting phase known as the light compensation point (LCP) instead of the traditional dark phase, and variations in the light spectrum impact the agricultural outcomes of organically cultivated leafy greens. In controlled growth chamber environments, a variety of leafy plant species (*Spinacia oleracea* L., *Ocimum basilicum*, *Beta vulgaris* L., *Lactuca sativa* L. cv. 'Garrison' and 'Blade', *Brassica rapa* cv. 'Japonica' and 'Chinensis', *Brassica juncea* cv. 'Scarlet Frills' and 'Wasabina', *Eruca sativa* and *Perilla frutescens* L.) were subjected to four light treatments with varying intensities and durations of lighting, while in a second experiment, five different spectral growing conditions were compared. Irrespective of the plant species, shortening the length of the diel cycle by extending the cumulative daily lighting to 20–24 h per day (5L/1N [5 h at 261 µmol m^{-2} s^{-1} + 1 h darkness for a total of 20 h of light per day] and 5L/1LCP [5 h at 256 µmol m^{-2} s^{-1} + 1 h LCP at 20 µmol m^{-2} s^{-1} for a total of 24 h of light per day]) led to an average increase of +12% in height, fresh weight (+16%), dry weight (+23%), and specific leaf weight (+11%), compared to the control plants (18L/6N; 18 h at 289 µmol m^{-2} s^{-1} + 6 h darkness) and 6L/6LCP plants (6 h at 418 µmol m^{-2} s^{-1} + 6 h LCP at 20 µmol m^{-2} s^{-1} for a total of 24 h of light per day) during the first harvest. This also resulted in better light utilization, expressed as increased fresh (+16%) and dry (+24%) biomass per mol of light received. Conversely, the studied light spectral treatments had no effect on the growth parameters of the four selected species. In conclusion, our study showed that reducing light intensity while extending the photoperiod could potentially represent a cost-effective LED strategy for the indoor cultivation of organically or conventionally grown leafy greens.

Keywords: vertical farming; artificial lighting; photoperiod; leafy vegetables

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1. Introduction

What is the optimal lighting strategy for both the indoor cultivation of leafy greens and enhancing the quality of organically grown plants? This question has remained unanswered for a considerable period, and this study aims to provide some insights by examining various strategies encompassing the photoperiod, photosynthetic photon flux density (PPFD; 400 nm to 700 nm), and the spectral light quality.

Lettuce, a leafy green species commonly grown in commercial vertical farming, can be produced under 24-h continuous lighting depending on the cultivars [1]. The diel cycle can even be fractionated without any physiological disorder [2,3]. Normally, the PPFD employed for the indoor cultivation of leafy greens falls within the range of 150 to 250 µmol m^{-2} s^{-1}, combined with a 16-h photoperiod [4]. Furthermore, lettuce is recognized for its high light use efficiency when exposed to a PPFD of 250 µmol m^{-2} s^{-1} and a

daily light integral (DLI) of 14.4 mol m^{-2} d^{-1} [5]. Likewise, Paz et al. [6] reported that lettuce necessitates a minimum DLI ranging from 6.5 to 9.7 mol m^{-2} d^{-1}, with a proportional rise in dry biomass observed when the DLI ranges from 8 to 22 mol m^{-2} d^{-1}. Nevertheless, tipburn may occur when the DLI exceeds 12 mol m^{-2} d^{-1} [7]. Our research involving leafy vegetables, such as lettuce and arugula [8,9], along with the findings reported by Hiroki et al. [10], have demonstrated that growers can adjust the diel cycle without compromising the yields as long as the DLI remains consistent. This flexibility enables them to tailor their lighting approach to their vertical growing system, thereby enhancing their economic gains (J. Eaves personal communication). In fact, a shorter dark period was observed to enhance lettuce growth, with the fresh weight of lettuce cultivated under a 16-h light/2-h dark cycle being approximately 30% greater than that of lettuce grown under a 16-h light/8-h dark cycle [10]. When comparing segmented photoperiods with a short (1-h) dark period and a short (1-h) or long (6-h) period of low light intensity equivalent to the light compensation point (LCP), we observed that, for the same DLI, one lettuce cultivar exhibited a higher shoot fresh weight under a 5-h photoperiod followed by 1 h of low light (5L/1LCP) compared to a 6-h photoperiod followed by 6 h of low light (6L/6LCP) [8]. The photon yield (g mol^{-1}) calculated as the plant fresh weight per mol of photons delivered during the cultivation period was higher under the conditions of a short night (5L/1N) or a period of low light intensity (5L/1LCP) when compared to a longer photoperiod and period of low light (6L/6LCP). Therefore, dividing the photoperiod into segments with the periods of low light intensity equivalent to the LCP may enable the utilization of a higher DLI while maintaining the same PPFD, thereby providing "growth breaks" to prevent physiological disorders. This new lighting strategy is expected to provide an optimal amount of light per day to various leafy green species, ensuring high light use efficiency and avoiding the occurrence of physiological disorders like tipburn.

Light conditions, including light quality, light intensity, and photoperiod, rank among the most crucial environmental factors influencing the growth, development, and accumulation of phytochemicals in vegetables, especially those cultivated in controlled environments [10–13]. For example, blue and red wavelengths are essential for photosynthesis because they correspond to the peak absorption wavelengths of chlorophyll [14]. Red light typically forms the foundation of horticultural lighting spectra due to its pivotal role in promoting plant growth [15]. Red light exerts a significant influence on plant biomass, and the red/far-red ratio triggers phytochrome photomorphogenic responses such as stem elongation and germination [16]. On the other hand, blue light is essential for enhancing growth while minimizing the shade avoidance responses induced by far-red light. Blue light typically restrains stem and leaf area growth and promotes stomatal opening [16,17]. Red light is also known to stimulate the synthesis of antioxidants, chlorophyll, and carotenoids [18,19], whereas blue light induces the production of anthocyanins and polyphenols, enhances the carotenoid content, and reduces the nitrate concentration, although these responses can vary depending on the specific plant species [4,20]. Green light was long regarded as inefficient for photosynthesis because it is absorbed to a lesser extent than the other wavelengths [21]. Recent studies, however, showed that transmitted green light in the lower canopy contributes significantly to photosynthesis and increases lettuce biomass [22,23]. Furthermore, when used in combination with far-red light, green light has been shown to trigger shade-avoidance responses to a greater extent than either far-red or green light alone [24]. This combination enhances light interception and fosters plant growth [16]. Using these wavelengths on indoor leafy greens, even though far-red is considered outside the range of photosynthetically active radiation (PAR), can lead to an increase in plant biomass and leaf length [25,26]. However, it may also have adverse effects on the chlorophyll, anthocyanin, and carotenoid concentrations [15].

In our current study, we posited that, under the same DLI of 18.8 mol m^{-2} day^{-1}, leafy greens subjected to alternating short photoperiods with high PPFD followed by an equivalent or shorter photoperiod of low PPFD, corresponding to their LCP, would exhibit superior growth, light use efficiency, and leaf quality attributes compared to plants culti-

vated under moderate PPFD conditions with a dark period. Additionally, we hypothesized that white broad-spectrum LED lighting would yield higher-quality leafy greens than LED lighting with only red and blue spectra. We also assumed that the indoor cultivation of leafy greens would exhibit productivity and quality on par with greenhouse cultivation. To assess these hypotheses, we investigated the impact of various lighting strategies under a similar DLI by substituting the dark phase with low-intensity lighting equivalent to the LCP. We also examined the influence of green and far-red light on various organically cultivated leafy green species, analyzing their effects on growth, yield, and quality. Furthermore, we conducted a comparative assessment of crop productivity and quality between the indoor-cultivated plants and those grown in a greenhouse setting.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

An experiment exploring the photoperiod and PPFD treatments (Photoperiod × PPFD) was conducted at Laval University (Quebec, QC, Canada) with 12 different leafy greens: spinach (*Spinacia oleracea* L.), basil (*Ocimum basilicum* cv. 'Citriodora' L.), Swiss chard (*Beta vulgaris* L.), chicory (*Cichorium endivia* L.), lettuce cv. 'Garrison' (*Lactuca sativa* L.), lettuce cv. 'Blade' (*Lactuca sativa* L.), mizuna (*Brassica rapa* cv. 'Japonica'), mustard cv. 'Scarlet Frills' (*Brassica juncea*), mustard cv. 'Wasabina' (*Brassica juncea*), pak choi (*Brassica rapa* cv. 'Chinensis), arugula (*Eruca sativa*), and shiso (*Perilla frutescens* L.) from Johnny's selected organic seeds (Winslow, ME, USA). This experiment took place in two controlled growth chamber environments (Conviron, Winnipeg, MB, Canada) and was repeated three times over a period of around 4 months. Each species was sown in 0.078 m^2 trays (27.94 cm × 27.94 cm) at a plant density of 4375 seeds m^{-2} and placed in the two growth chamber environments under their respective light treatments. The temperature, relative humidity, and CO_2 concentrations in the growth chambers were monitored and maintained at 21 ± 1 °C, 65% and 430 ppm, respectively. Each tray was filled with 800 g of an organic peat-based substrate (Premier Tech, Rivière-du-Loup, QC, Canada) enriched with compost, pelleted poultry manure, feather meal, and potassium sulphate to fulfill the fertilization needs of the species studied, according to the Biogreenhouse Guide Research [27]. Based on the specific leafy green species and previous experiments [28], two levels of organic fertilization were selected: the 100% level (14.1 g N m^{-2}, 1.9 g P m^{-2}, 9.8 g K m^{-2}, 9.0 g Ca m^{-2}, and 1.3 g Mg m^{-2}) for the lettuce cultivars, shiso, chicory, and basil, and the 150% level (21.1 g N m^{-2}, 2.9 g P m^{-2}, 14.8 g K m^{-2}, 13.6 g Ca m^{-2}, and 2.0 g Mg m^{-2}) for the other species. Two harvests were conducted, with the initial harvest occurring on the 21st day after sowing (DAS) for early-growing species like spinach, Swiss chard, lettuce cv. 'Garrison' and cv. 'Blade', pak choi, mizuna, mustard cv. 'Wasabina' and 'Scarlet Frills' as well as arugula. The second harvest took place on the 25th DAS for later-growing species including chicory, shiso, and basil. Additionally, a second cut was performed on the 29th DAS for the early-growing species and on the 32nd DAS for the later-growing species.

A second experiment on spectral quality was conducted at L'Abri Végétal (organic greenhouse producer, Compton, QC, Canada; 45°15′47.6″ N, 71°51′06.2″ W) in a commercially controlled growth chamber environment with an Argus control system (Argus Control Systems Limited, Surrey, BC, Canada) and was repeated three times over a period of time of around 4 months. Four species of leafy greens were studied: Swiss chard, pak choi, mizuna, and lettuce cv. 'Blade'. Plant growing conditions, density, and fertilization were the same as described for the Photoperiod × PPFD experiment performed at Laval University. Only one harvest was performed on the 21st DAS.

2.2. Light Treatments

For the Photoperiod × PPFD experiment conducted at Laval University, the plants were subjected to four treatments: (i) 5L/1N (5 h at 261 µmol m^{-2} s^{-1} + 1 h of darkness), (ii) 5L/1LCP (5 h at 256 µmol m^{-2} s^{-1} + 1 h in LCP at 20 µmol m^{-2} s^{-1}), (iii) 6L/6LCP (6 h at 418 µmol m^{-2} s^{-1} + 6 h in LCP at 20 µmol m^{-2} s^{-1}), and (iv) control 18L/6N

(18 h at 289 µmol m^{-2} s^{-1} + 6 h of darkness) (Table 1). A 24-h control treatment with 217.5 µmol m^{-2} s^{-1} was initially tested; however, it was discontinued due to the development of severe leaf chlorosis in two plant species, pak choi and arugula. Since the 18L/6N treatment is commonly used in indoor vertical agriculture [29], it served as the control treatment. The light intensity of the light compensation point (LCP) was set at 20 µmol m^{-2} s^{-1} based on reported average values from the scientific literature: lettuce had an LCP of ~17 µmol m^{-2} s^{-1} under HPS lamps [30] and ~30 µmol m^{-2} s^{-1} under LED lighting (R:B = 83:17%) [2]. A daily light integral (DLI) of 18.8 mol m^{-2} d^{-1} was used for all treatments and plant species. Light was provided via white broad-spectrum LEDs, model FN12 from Futur Vert (Prévost, QC, Canada). A homogeneity assessment of the light intensity was conducted during the installation of the lighting systems using an LI-191R Line Quantum Sensor and an LI-190R Quantum Sensor (Li-Cor Biosciences, Lincoln, NE, USA). This was performed to verify that the PPFD (400–700 nm) within the growing area matched the intended intensities for the studied treatments. For each time replicate, PPFD, PFD (380–780 nm), and the spectral quality were measured and validated in each experimental unit by using an LI-180 spectrometer (Li-Cor Biosciences, Lincoln, NE, USA).

Table 1. Light treatments of the Photoperiod × PPFD experiment (experiment 1) and the spectral quality experiment (experiment 2) conducted in controlled growth environments.

Treatments-Experiment 1	Duration of the Lighting Period per Cycle (h)	Duration of Dark Period per Cycle	Duration of the Lighting Period per Day (h)	Duration of Dark Period per Day	Light (L) PPFD (µmol m^{-2} s^{-1})	LCP PPFD (µmol m^{-2} s^{-1})	DLI (mol m^{-2} d^{-1})
5L/1N	5	1	20	4	261	0	18.79
5L/1LCP	6	0	24	0	256	20	18.72
6L/6LCP	12	0	24	0	418	20	18.92
18L/6N (ctrl)	18	6	18	6	289	0	18.73
Treatments-Experiment 2	**B (400–500 nm)**	**G (500–600 nm)**	**R (600–700 nm)**	**FR (700–780 nm)**	**[x] SL PPFD (µmol m^{-2} s^{-1})**	**SL Period per day (h)**	**SL DLI (mol m^{-2} d^{-1})**
CW	16%	40%	41%	3%	222	20	15.98
WW	14%	39%	44%	3%	219	20	15.77
RB	11%	2%	85%	2%	209	20	15.05
RB + FR	7%	1%	61%	31%	219	20	15.77
S + HPS (ctrl) *	3%	40%	47%	10%	201	≤20	≤14.47

[x] Supplemental lighting; * The supplemental HPS lighting (SL) was added to the solar natural greenhouse light when the solar radiation fell below 250 W m^{-2} (45°15′47.6″ N, 71°51′06.2″ W).

The second experiment regarding the spectral quality compared five treatments with the following spectral composition (in %): (i) cold white (CW) B:G:R:FR of 16:40:41:3; (ii) warm white (WW) B:G:R:FR of 14:39:44:3; (iii) red blue (RB) B:G:R:FR of 11:2:85:2; (iv) red blue + far-red (RB + FR) B:G:R:FR of 7:1:61:31, and (v) control sunlight + HPS (S + HPS), HPS B:G:R:FR of 3:40:47:10 (Table 1). The PPFD of the different treatments in the growth chamber varied between 209 and 222 µmol m^{-2} s^{-1} with a 20-h photoperiod. The natural solar radiation + HPS treatment was placed on rolling benches inside the greenhouse at L'Abri Végétal. While the greenhouse control treatment (S + HPS) exhibited variability in the climatic growing conditions compared to the controlled growth chamber environment, we included this treatment intentionally in our experimental design to facilitate a comparison with the indoor lighting treatments. Experiments were performed from 24 March to 8 August 2020. The PPFD delivered using the HPS lamps was 201 µmol m^{-2} s^{-1}, while the photon flux density (PFD) ranging from 380 to 780 nm was 232 µmol m^{-2} s^{-1}. The HPS lamps were turned on when solar radiation fell below 250 W m^{-2} and turned off upon reaching a cumulative daily energy input of 950 joules cm^{-2}. Table 1 outlines the spectral quality distribution, with PPFD and DLI supplied using LED or HPS lamps for each treatment. FN12 LEDs from Futur Vert (Prévost, QC, Canada) were employed for the CW and WW treatments, whereas GreenPower lamps from Philips (Eindhoven, North Brabant, Netherlands) were utilized for the RB and RB+FR treatments. HPS 615W lamps from Agrolux (Rozenburg, South Holland, Netherlands) were employed to illuminate the

control treatment. The measurements and validation of the homogeneity of PPFD, PFD, and spectral quality uniformity were conducted using the LI-180 spectrometer from LICOR (Li-Cor Biosciences, Lincoln, NE, USA).

2.3. Measured Parameters

2.3.1. Growth Parameters and Photon Yield

For both experiments, the measured growth parameters were the average height of the plants in each tray (three diagonal measurements per tray with each tray being an experimental unit), the total weight of shoot fresh weight (SFW) on a crop area basis (kg FW m^{-2}), shoot dry weight (SDW) per crop area unit (g DW m^{-2}) after oven-drying at 65 °C for 3 days, and then the percentage of the water content (%) was calculated as $1 - (DW \times 100)/FW$ [8]. The leaf area was measured immediately after cutting with a LI-3100C Leaf Area Meter (Li-Cor Biosciences, Lincoln, NE, USA) to calculate the specific leaf area (SLA; cm^2 g^{-1} DW), the specific leaf weight (SLW; mg DW cm^{-2}), and the leaf area index (cm^2 of leaves per cm^2 of the floor). Leaf area measurements were not taken for the commercial trials. Photon yield (PY), an indicator of the plant's light use performance, was calculated as the cumulative fresh and dry biomass divided by the cumulative photon flux density, and expressed as g FW mol^{-1} photons and g DW mol^{-1} photons [8].

2.3.2. Photobiological Parameters

In the Photoperiod × PPFD experiment, photobiological parameters were measured the day before harvesting, one hour after the first daily irrigation. Chlorophyll fluorescence parameters were measured with a Handy PEA fluorimeter (Hansatech Instruments, Pentney, Norfolk, UK). Dark adaptation clips were placed on three well-developed leaves per experimental unit, randomly selected from three distinct plants while avoiding the central vein. This was conducted for the duration of 15 to 20 min before conducting the measurements, following the guidelines recommended by Hansatech and previously validated. The F_V/F_M ratio assessed the photosynthetic performance of the plant, more precisely the efficiency of photosystem II, while the performance index (PI) is an indicator of the vitality of the sample and its ability to resist external constraints [31].

The parameters were calculated according to the following equations [32]:

$$F_V/F_M = (F_M - F_0)/F_M \quad (1)$$

$$PI = (1 - (F_0/F_M))/(M_0/V_J) \times (F_M - F_0)/F_0 \times (1 - V_J)/V_J \quad (2)$$

where F_0 = fluorescence intensity at 50 µs; $F_{300\mu s}$ = fluorescence intensity at 300 µs; F_J = fluorescence intensity at the J step (at 2 ms); F_M = maximal fluorescence intensity; V_J = relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_0)/(F_M - F_0)$; and M_0 = initial slope of fluorescence kinetics, which can be derived from the equation:

$$M_0 = 4 \times ((F_{300\mu s} - F_0)/(F_M - F_0)) \quad (3)$$

The leaf chlorophyll content (SPAD unit) was determined using the SPAD-502 Plus chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan). Measurements were taken on three well-developed leaves per experimental unit, which had previously been used to record the chlorophyll fluorescence parameters. Around one to two hours elapsed between both measurements. The SPAD unit is an indicator of the chlorophyll content and is closely correlated with the nitrogen nutritional status of the plant [33,34].

Photosynthetic light response curves were conducted using a LI-6400XT (Li-Cor Biosciences, Lincoln, NE, USA) on Swiss chard, lettuce cv. 'Garrison', pak choi, and mizuna, with one response curve generated for each experimental unit (e.u.). Measurements were exclusively conducted on species that had leaves of a size suitable for placement within the 2 × 3 cm leaf chamber. Leaf chamber parameters were set to maintain airflow at 350 µmol s^{-1}, with the leaf temperature at 21 °C, a sample CO_2 concentration of 400 ppm, and a vapor pressure deficit (VDP) between 0.8 and 0.9 kPa. These settings were chosen to replicate the growing conditions within the controlled growth chambers. An automated

light response curve sequence was programmed with 15 different light intensities: 1800, 1800, 1500, 1200, 900, 700, 550, 375, 275, 200, 150, 100, 75, 50, 20, and 1 µmol m^{-2} s^{-1}. Two measurements of photosynthesis were taken at 1800 µmol m^{-2} s^{-1} to allow the plants to acclimate to a much higher photosynthetic photon flux density (PPFD) than the one used in the experimental treatments. Subsequently, the other parameters, including Amax (µmol CO_2 m^{-2} s^{-1}) representing the maximum rate of net photosynthesis, Rd (µmol CO_2 m^{-2} s^{-1}) representing the rate of respiration in the dark, and ϕ (µmol CO_2 µmol $photons^{-1}$) representing the apparent quantum yield, were computed from the photosynthetic light response curves using the hyperbolic model of Hansen et al. [35]. The light compensation point (LCP) and the light saturation point (LSP) (µmol photon m^{-2} s^{-1}) were estimated from these curves.

2.3.3. Leaf Quality Parameter

In both experiments, the concentrations of total polyphenols, anthocyanins, and nitrates were measured in the harvested biomass. The samples (8 to 10 g FW/e.u.) that were randomly collected from the first harvest were frozen, lyophilized, and then analyzed. The total polyphenol concentration (TPC) was measured by using the Folin–Ciocalteu (F-C) method adapted from Singleton and Rossi [36]. A Folin–Ciocalteu reagent made up of sodium carbonate solution, gallic acid, and 80% methanol was prepared. The lyophilized samples from each experimental unit were crushed by hand with a pestle and 0.05 g of powder from each sample was placed into two 50 mL Falcon tubes to have two lab replicas per sample (a total of six analyses per treatment). The powder was mixed with 5 mL of methanol 80% with a vortex for 30 s and placed in an ultrasonic bath at 37 °C for 25 min. After centrifuging at 4000 rpm for 4 min, the supernatant was transferred to another 50 mL Falcon tube. The extraction was repeated a second time, with 3 mL of 80% methanol and 25 min in the ultrasonic bath. The extraction was then completed with water. Standard solutions, consisting of four concentrations of gallic acid (200, 100, 50, and 25 ppm) were prepared. The control (20 µL of water), the standard solutions, and the various samples were placed in a microplate and were mixed with 100 µL of the Folin–Ciocalteu reagent to carry out the reaction. After that, 80 µL of the 7.5% sodium carbonate solution (Na_2CO_3) was added. Then, the microplate was mixed and left to stand in the dark for 45 min. Absorption was measured at 765 nm using an Epoch 2 microplate reader spectrophotometer (BioTek Instruments, Inc. Winooski, VT, USA).

The anthocyanin concentration was determined via the pH differential method adapted from Lee et al. [37]. The extraction was carried out using a solvent composed of methanol, water, and acetic acid (85:15: 0.5 v/v, MeOH/H_2O/AcOH), according to Wu and Prior [38]. For the extraction, 0.5 g of powder from each sample was placed in 50 mL Falcon tubes, with two lab replicates per sample (a total of six analyses per treatment). The two reagents were prepared: a pH 1.0 buffer (potassium chloride, 0.025 M) and a pH 4.5 buffer (sodium acetate, 0.4 M). Then, 5 mL of acidic methanol solvent was added to the tubes and mixed with a vortex for 30 s. The tubes were placed in the ultrasonic bath for 20 min and were transferred to the centrifuge for 4 min at 4000 rpm. After centrifugation, the supernatant was transferred to a new 50 mL Falcon tube. The extraction was repeated three times to obtain a homogenized extract. To determine the dilution factor, a colorimetric test was conducted using the pH 1.0 and pH 4.5 buffers. Blanks were made with the pH 1.0 and pH 4.5 buffers. A volume of 0.5 mL of diluted extract and 2.5 mL of buffers were added to the 4 mL cuvettes of the microplate. The solution was well mixed and left for 30 min at room temperature. Then, the absorption was measured at 510 nm and 700 nm using an Epoch 2 microplate reader spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The shoot nitrate content was extracted in water (45 °C) according to the method developed by Cataldo et al. [39], and then quantified by using high-performance liquid chromatography (Water Chromatography Division, Milford, MA, USA) and UV absorption (214 nm) with a PDA detector model 996, using the method #A-107 and borate–gluconate as the eluent.

2.4. Statistical Analysis

The data were analyzed using an analysis of variance (ANOVA) and the means were compared with protected LSD and the t-test for multiple comparison at a 5% significance level. Analysis of variance (ANOVA) was performed using the MIXED procedure of SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). The postulates were respected, the homogeneity of variance was validated using the Shapiro–Wilk test and the normality via a plot of the residuals. Statistical tests were carried out on the original data, except for the photosynthetic response curves where the Hansen equation allowed us to extract the photosynthetic parameters (Amax, Rd, ϕ, LCP, LSP).

3. Results

3.1. Growth Parameters and Photon Yield

For the measured growth parameters, there was no significant interaction detected between the species and lighting treatments, as indicated in Table 2. However, exceptions were observed for the plant dry weight during the second harvest in the Photoperiod × PPFD experiment (Table 3), as well as for the shoot water content and plant height in the spectral quality experiment (Table 4). It is noteworthy that no physiological disorders were observed in these experiments.

Table 2. Agronomic parameters for the first harvest of 12 leafy green species cultivated in controlled growth chamber environments and submitted to four photoperiod and PPFD lighting treatments (Photoperiod × PPFD experiment). The data represent the mean values of three experimental replicates per treatment (n = 3).

	SFW (kg FW m^{-2})	SDW (g DW m^{-2})	Water Content (%)	Height (cm)	SLA (cm^2 g^{-1} DW)	SLW (mg DW cm^{-2})	LAI	PY (FW) (g FW mol^{-1} Photons)	PY (DW) (g DW mol^{-1} Photons)
SPECIES									
Spinach	1.44 ef [x]	112.3 cd	92.1 cd	9.9 d	319.9 g	3.15 a	3.64 f	3.71 ef	0.29 de
Swiss chard	1.72 cd	99.9 fg	94.1 g	10.0 d	416.9 c	2.44 e	4.20 e	4.43 cd	0.26 f
Chicory	1.59 def	90.4 gh	94.3 g	8.6 g	442.4 b	2.28 f	4.07 e	3.43 f	0.20 g
Shiso	0.69 h	92.6 fg	86.9 a	9.2 ef	368.9 d	2.73 d	3.44 f	1.48 h	0.20 g
Basilic	0.92 g	76.6 h	91.7 c	7.9 h	370.0 de	2.77 cd	2.64 g	1.99 g	0.17 h
L. Garrison	2.19 a	98.0 efg	95.5 i	11.3 b	779.1 a	1.33 g	7.72 b	5.65 a	0.25 f
Pak choi	1.63 def	104.4 def	93.5 f	8.9 fg	423.1 bc	2.41 ef	4.47 de	4.21 d	0.27 ef
Mizuna	2.06 ab	180.7 a	91.1 b	13.0 a	310.6 g	3.32 a	5.66 c	5.31 ab	0.47 a
M. Wasabina	1.87 bc	139.8 b	92.4 d	9.7 de	344.4 ef	2.94 bc	4.86 d	4.82 bc	0.36 b
M. Scarlett Frills	1.55 def	111.8 de	92.7 e	11.4 b	326.4 fg	3.09 ab	3.66 f	3.99 de	0,29 de
L. Blade	2.24 a	118.8 cd	94.6 h	11.8 b	739.5 a	1.40 g	8.86 a	5.78 a	0.31 cd
Arugula	1.42 f	126.1 c	90.9 b	10.7 c	315.5 g	3.24 ab	4.05 e	3.66 f	0.33 bc
TREATMENTS [y]									
5L/1N	1.70 a	121.6 a	92.4 b	10.7 a	410.3 b	2.68 a	4.88 ab	4.27 a	0.30 a
5L/1LCP	1.76 a	127.2 a	92.2 b	10.9 a	416.6 b	2.69 a	5.24 a	4.40 a	0.32 a
6L/6LCP	1.55 b	101.0 b	92.9 a	9.8 b	456.1 a	2.42 b	4.62 bc	3.89 b	0.25 b
18L/6N (ctr)	1.43 b	100.7 b	92.5 ab	9.4 b	436.0 ab	2.59 a	4.35 c	3.59 b	0.25 b
					ANOVA—*p* values				
Species (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatments (T)	0.002	<0.001	0.021	<0.001	0.010	0.008	0.006	0.003	<0.001
S × T	0.984	0.399	0.158	0.076	0.425	0.333	0.298	0.932	0.303

[x] means of the same column with different letters are significantly different at $p \leq 0.05$ (Fisher's protected LSD). [y] lighting treatments: 5L/1N, 5 h at 261 µmol m^{-2} s^{-1} + 1 h of darkness; 5L/1LCP, 5 h at 256 µmol m^{-2} s^{-1} + 1 h at LCP of 20 µmol m^{-2} s^{-1}; 6L/6LCP, 6 h at 418 µmol m^{-2} s^{-1}+ 6 h at LCP of 20 µmol m^{-2} s^{-1}; 18L/6N control, 18 h at 289 µmol m^{-2} s^{-1} + 6 h of darkness.

In the Photoperiod × PPFD experiment, as anticipated, variations between species were noted for all studied growth parameters, as outlined in Tables 2 and 3. Among the species, lettuce exhibited the highest fresh biomass during the first harvest, followed by mizuna, mustard cv. 'Wasabina', Swiss chard, pak choi, chicory, mustard cv. 'Scarlett Frills', spinach, arugula, shiso, and basil. In the second growth cycle, which occurred after the first harvest, spinach and arugula started flowering due to the stress induced by the initial cut. Trichomes were also observed on the inner side of the spinach leaves. Basil and shiso did not adapt well to a second cut, as the stems remaining from the first harvest were included in the

second harvest. Given these observations and the subpar performance of these last species after the first harvest, the results for their second harvest are not considered in Table 3.

Table 3. Agronomic parameters for the second harvest of eight leafy green species cultivated in controlled growth chamber environments and submitted to four photoperiod and PPFD lighting treatments (Photoperiod × PPFD experiment). The data represent the mean values of three experimental replicates per treatment (n = 3).

	SFW (kg FW m^{-2})	SDW (g DW m^{-2})	Water Content (%)	Height (cm)	SLA (cm^2 g^{-1} DW)	SLW (mg DW cm^{-2})	LAI	PY (FW) (g FW mol^{-1} Photons)	PY (DW) (g DW mol^{-1} Photons)
SPECIES									
Swiss chard	1.55 a [x]	93.0 a	91.8 bc	7.82 bc	263.8 c	4.03 bc	2.95 b	2.88 a	0.18 ab
Chicory	0.95 d	61.4 d	93.0 ab	7.87 c	440.6 b	2.35 d	2.74 bc	1.59 d	0.11 e
L. Garrison	1.32 bc	67.5 cd	93.9 a	6.90 de	498.3 a	2.12 e	3.78 a	2.46 bc	0.13 de
Pak choi	1.41 ab	84.2 ab	92.5 bc	6.48 e	260.5 c	3.87 c	2.58 bc	2.64 ab	0.16 bcd
Mizuna	1.26 c	111.1 a	89.1 d	9.69 a	192.9 d	5.23 a	2.43 c	2.34 c	0.21 a
M. Wasabina	1.34 bc	107.2 a	89.9 d	7.01 d	207.7 d	5.06 ab	2.57 bc	2.51 bc	0.20 a
M. Scarlett Frills	1.31 bc	85.9 abc	91.4 c	8.48 b	204.0 d	5.12 a	2.16 d	2.44 bc	0.16 bc
L. Blade	1.20 c	69.7 bcd	93.0 ab	8.10 bc	457.9 b	2.27 de	3.63 a	2.24 c	0.13 cde
TREATMENTS									
5L/1N [y]	1.35	93.0	91.7	8.10 a	313.5	3.84	2.97	2.50	0.17
5L/1LCP	1.32	91.2	91.3	8.39 a	295.7	4.13	2.84	2.44	0.17
6L/6LCP	1.23	74.5	92.4	7.33 b	316.3	3.71	2.71	2.35	0.15
18L/6N (ctr)	1.27	81.3	91.9	7.36 b	337.3	3.35	2.91	2.27	0.15
ANOVA—*p* values									
Species (S)	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatments (T)	0.488	0.284	0.170	0.003	0.127	0.183	0.376	0.488	0.287
S × T	0.882	0.022	0.976	0.820	0.272	0.692	0.514	0.881	0.968

[x] means of the same column with different letters are significantly different at $p \leq 0.05$ (Fisher's protected LSD). [y] lighting treatments: 5L/1N, 5 h at 261 μmol m^{-2} s^{-1} + 1 h of darkness; 5L/1LCP, 5 h at 256 μmol m^{-2} s^{-1} + 1 h at LCP of 20 μmol m^{-2} s^{-1}; 6L/6LCP, 6 h at 418 μmol m^{-2} s^{-1} + 6 h at LCP of 20 μmol m^{-2} s^{-1}; 18L/6N control, 18 h at 289 μmol m^{-2} s^{-1} + 6 h of darkness.

Table 4. Agronomic parameters of four leafy green species cultivated in a commercial controlled growth chamber environment and submitted to five spectral lighting treatments. The data represent the mean values of three experimental replicates per treatment (n = 3).

	SFW (kg FW m^{-2})	SDW (g DWm^{-2})	Water Content (%)	Height (cm)	Anthocyanins (mg g^{-1} DW)	Polyphenols (mg g^{-1} DW)	Nitrate (mg g^{-1} DW)	PY (FW) (g FW mol^{-1} Photons)	PY (DW) (g DW mol^{-1} Photons)
SPECIES									
Swiss chard	2.22 c [x]	129.9 b	93.6	11.4	nd [y]	6.32 d	2.85	6.47 c	0.38 b
Pak choi	2.71 ab	138.8 b	94.6	10.3	nd	8.14 b	5.52	7.68 bc	0.39 b
Mizuna	2.68 b	201.5 a	92.1	14.1	nd	7.19 c	3.48	8.33 b	0.63 a
L. Blade	3.04 a	142.2 b	95.1	13.5	0.157	18.89 a	3.35	9.46 a	0.44 b
TREATMENTS									
CW 16:41:40:3 [z]	2.77 a	180.7	93.1	11.8	0.184	10.44	2.23	8.08	0.54
WW 14:39:44:3	2.82 a	165.3	93.6	12.6	0.200	10.78	2.27	8.27	0.48
RB 11:2:85:2	2.77 a	138.1	94.6	11.2	0.215	10.64	2.68	8.16	0.40
RB + FR 7:1:61:31	2.90 a	161.2	94.1	14.4	0.133	8.65	4.94	9.00	0.50
S + HPS (ctr)	2.07 b	120.3	93.9	11.7	0.053	10.19	6.89	6.42	0.37
ANOVA—*p* values									
Species (S)	<0.001	0.003	<0.001	<0.001	-	<0.001	0.140	<0.001	0.001
Treatments (T)	0.052	0.094	0.090	0.517	0.668	0.544	0.276	0.163	0.175
S × T	0.109	0.176	0.021	0.034	-	0.074	0.934	0.103	0.110

[x] means of the same column with different letters are significantly different at $p \leq 0.05$ (Fisher's protected LSD), [y] not detectable. [z] lighting treatments: CW 16:41:40:3, Cold White with spectral composition of 16% Blue (B)—40% Green (G)—41% Red (R)—3% Far-Red (FR); WW 14:39:44:3, Warm White with 14% B—39% G—44% R—3% FR; RB 11:2:85:2, Red Blue treatment with 11% B—2% G—85% R—2% FR; RB + FR 7:1:61:3, Red Blue + Far-Red treatment with 7% B—1% G—61% R—31% FR; S + HPS control, Sunlight + HPS with spectral composition of the HPS lamp of 3% B—40% G—47% R—10% FR.

With the exception of the SLW, which exhibited an increase (averaging +44%), the values of the measured growth parameters were reduced during the second harvest for all species across all Photoperiod × PPFD treatments (Table 3). Among the lighting treatments, only plant height showed a significant difference, with 5L/1N and 5L/1LCP showing

higher values (+10.5% and +14.5%, respectively) compared to 6L/6LCP and 18L/6N. Regarding SDW, a significant interaction was observed between the light treatments and species. Specifically, lettuce cv. 'Garrison' exhibited a higher SDW under 5L/1N compared to the other treatments. As anticipated, there was a significant difference between species for all the studied growth parameters. Swiss chard displayed the highest fresh shoot biomass, followed by pak choi, mustard cv. 'Wasabina', lettuce cv. 'Garrison', 'Scarlett Frills', mizuna, lettuce cv. 'Blade', and chicory (Table 3).

Regardless of the species, the plants cultivated under 5L/1N and 5L/1LCP conditions achieved significantly higher fresh shoot biomass (an increase of 16%) and dry shoot biomass (an increase of 23%) compared to those grown under the 6L/6LCP and 18L/6N treatments (Table 2). Specifically, the 5L/1N and 5L/1LCP treatments led to a 19% and 23% increase in SFW, respectively, in comparison to the control treatment (18L/6N). Additionally, they resulted in a 10% and 13.5% higher SFW, respectively, compared to the 6L/6LCP treatment. Similarly, SDW exhibited higher values under the 5L/1N and 5L/1LCP treatments, with increases of 21% and 26% compared to the 18L/6N treatment and increases of 20% and 26% compared to the 6L/6LCP treatment, respectively. There was a slight decrease in the water content for the 5L/1N (–0.5%) and 5L/1LCP (−0.8%) treatments compared to the 6L/6LCP treatment. Plant height showed a significant increase under the 5L/1N and 5L/1LCP treatments (averaging +12.5%; ranging from 9% to 16%) compared to the 18L/6N and 6L/6LCP treatments. Additionally, SLA was significantly higher under the 6L/6LCP treatment (+10.3%) than under the 5L/1N and 5L/1LCP treatments, while there was no significant difference compared to the control treatment (18L/6N). SLW was significantly lower under the 6L/6LCP treatment (−9%) compared to the other treatments. LAI reached its highest value under the 5L/1LCP treatment (+20.5% compared to 18L/6N and +13% compared to 6L/6LCP), but it was similar to the 5L/1N treatment.

Photon yields exhibited the same trend as biomass and were higher under the 5L/1N and 5L/1LCP treatments. Specifically, based on fresh weight, PY of 5L/1N and 5L/1LCP were higher (+19 % and +22.5%, respectively) compared to the control treatment of 18L/6N and by +10% and +13% compared to the 6L/6LCP treatment, respectively. Similarly, when considering the shoot dry weight, photon efficiency was, on average, greater under 5L/1N and 5L/1LCP (+24%) compared to 6L/6LCP and 18L/6N. There was no significant difference in photon yield between the 6L/6LCP and 18L/6N treatments.

In the spectral quality experiment, as anticipated, significant effects were observed among species for shoot fresh weight, shoot dry weight, leaf polyphenol concentration, and photon yield (Table 4). Moreover, significant interactions were observed between species and the lighting treatments for the shoot water content and plant height. However, photon yield expressed in terms of fresh or dry shoot biomass was not influenced by any of the spectral quality treatments. Although the measurements of the leaf area parameters were not performed for this experiment, visual observations revealed that Swiss chard and mizuna cultivated under the RB + FR treatment exhibited distinct morphological characteristics compared to the RB treatment, with cotyledons and leaves appearing much larger. Despite this visual difference, there were no statistically significant effects on SFW and SDW between RB+FR and the other treatments (CW, WW, and RB). In comparison to commercial greenhouse growing conditions (solar radiation [S] + HPS), the plants cultivated in the controlled growth chamber environments exhibited a higher fresh biomass (+36%). However, this difference was only significant at $p = 0.052$ (Table 4). Lettuce cv. 'Blade' achieved a greater plant height under RB+FR compared to RB alone. Among the treatments, Mizuna had the lowest water content in both the CW and WW treatments, while lettuce cv. 'Blade' had the highest water content under the control RB+FR treatment and the lowest water content under the S + HPS conditions.

3.2. Photobiological Parameters

In the first Photoperiod × PPFD experiment, as anticipated, significant differences were observed among plant species. Amax was higher for pak choi, followed by mizuna,

Swiss chard, and lettuce cv. 'Garrison', while Rd was higher for Swiss chard and pak choi, followed by mizuna and lettuce cv. 'Garrison'. The ϕ was lower for mizuna compared to Swiss chard and cv. 'Garrison', while the LCP for the latter last two species was higher than that of mizuna and pak choi (Table 5).

Table 5. Photobiological and quality parameters for the first harvest of 12 leafy green species cultivated in controlled growth chamber environments and submitted to four photoperiod and PPFD lighting treatments (photoperiod x PPFD experiment). The data represent the mean values of three experimental replicates per treatment (n = 3).

	Chlorophyll Content	F_V/F_M	PI	Amax	Rd	ϕ	LCP	LSP	Anthocyanins	Polyphenols	Nitrate
	(SPAD Unit)			(μmol CO_2 m^{-2} s^{-1})		(μmol CO_2 μmol $Photon^{-1}$)	(μmol Photon m^{-2} s^{-1})		(mg g^{-1} DW)		
SPECIES											
Spinach	34.4	0.800	4.46	-	-	-	-	-	nd	5.4	2.08
Swiss chard	32.5	0.822	5.65	14.33 b [x]	-1.35 b	0.096 a	14.84 a	1050	nd	7.3	2.93
Chicory	13.4	0.843	4.76	-	-	-	-	-	nd	5.5	3.88
Shiso	48.4	0.817	5.36	-	-	-	-	-	5.94 a	25.9	1.54
Basilic	31.6	0.785	1,54	-	-	-	-	-	0.06 b	21.8	4.93
L. Garrison	19.5	0.836	3.28	8.56 c	−0.81 a	0.090 a	9.30 b	670	0.50 b	15.5	4.90
Pak choi	47.7	0.820	7.58	18.52 a	−1.17 b	0.086 ab	13.99 a	1200	0.18 b	10.2	2.94
Mizuna	39.0	0.823	6.89	15.74 b	−0.84 a	0.082 b	10.58 b	1275	nd	7.7	0.51
M. Wasabina	33.5	0.830	7.46	-	-	-	-	-	nd	6.8	1.68
M. Scarlett Frills	32.6	0.818	6.13	-	-	-	-	-	0.06 b	8.7	2.50
L. Blade	28.0	0.824	3.48	-	-	-	-	-	0.32 b	25.1	2.63
Arugula	45.7	0.833	7.50	-	-	-	-	-	nd	7.8	1.83
TREATMENTS											
5L/1N [y]	33.1	0.818	5.08	16.08	−0.95	0.084	11.68 ab	1150	2.28	12.8	2.06
5L/1LCP	34.6	0.820	4.99	14.34	−1.03	0.089	11.18 b	970	2.09	12.6	2.28
6L/6LCP	33.4	0.828	6.13	12.21	−0.97	0.087	11.37 b	1000	1.92	11.7	3.61
18L/6N (ctr)	34.2	0.819	5.16	14.53	−1.22	0.092	14.48 a	1075	2.33	12.1	2.99
						ANOVA—*p* values					
Species (S)	<0.001	<0.001	<0.001	<0.001	<0.001	0.041	0.002	–	<0.001	<0.001	<0.001
Treatments (T)	0.087	0.183	0.217	0.456	0.327	0.803	0.054	–	0.567	0.543	0.031
S × T	0.010	<0.001	<0.001	0.173	0.672	0.504	0.487	–	0.257	<0.001	0.002

Note: -, not measured; –, estimated not repeated; nd, not detectable. [x] means of the same column with different letters are significantly different at $p \leq 0.05$ (Fisher's protected LSD) [y] lighting treatments: 5L/1N, 5 h at 261 µmol m^{-2} s^{-1} + 1 h of darkness; 5L/1LCP, 5 h at 256 µmol m^{-2} s^{-1} + 1 h at LCP of 20 µmol m^{-2} s^{-1}; 6L/6LCP, 6 h at 418 µmol m^{-2} s^{-1} + 6 h at LCP of 20 µmol m^{-2} s^{-1}; 18L/6N control, 18 h at 289 µmol m^{-2} s^{-1} + 6 h of darkness.

During the first growth cycle (Table 5), significant interactions between species and the lighting treatments were observed for the SPAD index, F_V/F_M, and performance index. The lowest SPAD values were observed under the 5L/1N treatment for Swiss chard and under the 6L/6LCP treatment for basil, while the highest values were observed under the 5L/1LCP treatment for Swiss chard, chicory, pak choi, and arugula. The F_V/F_M ratio was higher under the 6L/6LCP treatment for spinach, lettuce cv. 'Garrison', and cv. 'Blade' as well as mustard cv. 'Scarlet Frills', in comparison to the other lighting treatments and species. The highest PI was observed under the 6L/6LCP treatment for spinach, Swiss chard, lettuce cv. 'Garrison', pak choi, mustard cv. 'Scarlett Frills', and arugula. For chicory, the highest PI was achieved under the control 18L/6N treatment. No significant differences were found between the light spectral quality treatments for the other photobiological parameters, including Amax, Rd, ϕ, and LCP. However, at $p = 0.054$, the LCP of plants in the control 18L/6N treatment was higher (+27 to 29.5%) compared to the 5L/1LCP and 6L/6LCP plants.

For the second growth cycle (second harvest), the species had significant effects on all measured parameters, while significant light treatment effects were observed for F_V/F_M and PI, but not for the leaf chlorophyll content expressed via the SPAD index (Table 6). Specifically, the F_V/F_M ratio under the 6L/6LCP and 5L/1LCP treatments was slightly

but significantly higher (+1.1%) compared to the 5L/1N treatment. Additionally, the PI under the 6L/6LCP treatment was higher (+21%) compared to the 5L/1N and control 18L/6N treatments.

Table 6. Photobiological parameters for the second harvest of eight leafy green species cultivated in controlled growth chamber environments and submitted to four photoperiod and PPFD lighting treatments (Photoperiod × PPFD experiment). The data represent the mean values of three experimental replicates per treatment (n = 3).

	Chlorophyll Content (SPAD Unit)	F_V/F_M	PI
SPECIES			
Swiss chard	33.8 c [x]	0.807 e	3.2 e
Chicory	13.1 f	0.846 a	2.9 e
L. Garrison	23.6 e	0.845 a	6.2 cd
Pak choi	49.5 a	0.823 d	9.1 a
Mizuna	37.2 b	0.833 bc	7.0 bc
M. Wasabina	34.5 bc	0.833 bc	7.1 b
M. Scarlett Frills	29.7 d	0.827 cd	6.6 bcd
L. Blade	31.7 d	0.835 b	5.7 d
TREATMENTS			
5L/1N [y]	31.7	0.825 b	5.6 b
5L/1LCP	31.9	0.832 a	6.0 ab
6L/6LCP	31.7	0.836 a	6.8 a
18L/6N (ctr)	31.3	0.831 ab	5.6 b
	ANOVA—*p* values		
Species (S)	<0.001	<0.001	<0.001
Treatments (T)	0.937	0.050	0.026
S × T	0.268	0.172	0.423

[x] means of the same column with different letters are significantly different at $p \leq 0.05$ (Fisher's protected LSD).[y] lighting treatments: 5L/1N, 5 h at 261 μmol m^{-2} s^{-1} + 1 h of darkness; 5L/1LCP, 5 h at 256 μmol m^{-2} s^{-1} + 1 h at LCP of 20 μmol m^{-2} s^{-1}; 6L/6LCP, 6 h at 418 μmol m^{-2} s^{-1} + 6 h at LCP of 20 μmol m^{-2} s^{-1}; 18L/6N control, 18 h at 289 μmol m^{-2} s^{-1} + 6 h of darkness.

3.3. Leaf Quality Parameters

In the Photoperiod × PPFD experiment, significant interactions between the species and lighting treatments were observed for the leaf total polyphenol and nitrate concentrations. Shiso had the highest anthocyanin concentration compared to the other species (Table 5). The total polyphenol concentration was higher under the 6L/6LCP treatment for chicory and pak choi, while it was higher under the 18L/6N treatment for spinach. Arugula and mustard cv. 'Wasabina' achieved a higher total polyphenol concentration under both the 6L/6LCP and 18L/6N treatments. Spinach, shiso, basil, and mustard cv. 'Scarlett Frills' had the highest nitrate concentration under the 6L/6LCP treatment, while for lettuce cv. 'Garrison', it was under the control 18L/6N treatment. Swiss chard had the lowest nitrate concentration under the 5L/1LCP treatment.

In the spectral quality experiment, differences between species were observed for the total polyphenol concentration, but not for their nitrate concentration (Table 4). On the other hand, no significant effect of the lighting treatments was observed regarding the assessed quality attributes, indicating that the quality of indoor-cultivated leafy greens was comparable to that of greenhouse-grown plants. However, it is noteworthy that mizuna, Swiss card, and pak choi had a lower polyphenol concentration under the RB+FR treatment compared to the other treatments, although this difference was not statistically significant ($p = 0.074$).

4. Discussion

4.1. Effect of Plant Species

As anticipated, significant differences were observed between the studied species for all the studied parameters, except for the leaf nitrate concentration in the spectral quality experiment. Furthermore, significant differences between cultivars were observed for some of the parameters.

4.2. Effect of Segmenting and Lengthening the Photoperiod for a Same DLI

This study clearly demonstrates the benefits of segmenting and extending the photoperiod (from 12 and 18 h to 20 h per day) while maintaining the same DLI for the twelve organically cultivated leafy greens studied, particularly in terms of plant biomass. These findings align with previous studies conducted on lettuce [2,3,8,9], suggesting that this lighting strategy can be advantageous for various leafy greens. Interestingly, the use of a 24-h photoperiod at 217.5 µmol m^{-2} s^{-1} had a negative impact on the growth of only two species, pak choi and arugula. In contrast to our findings, Hiroki et al. [10] reported that continuous lighting was not desirable for growing lettuce. They observed a decline in the rate of photosynthesis, primarily due to the accumulation of photosynthetic carbohydrates, followed by changes in transpiration rates, which led to the development of marginal necrosis (tipburn).

Despite the potential risk of physiological disorders such as tipburn when the DLI exceeds 12 mol m^{-2} d^{-1} [7], the twelve leafy green species or cultivars investigated in this study performed well under a DLI ranging from 13 to 18.8 mol m^{-2} d^{-1}. Similarly, Brechner and Both [40] observed favorable growth in lettuce, spinach, arugula, and kale under a DLI of 17 to 22 mol m^{-2} d^{-1}. According to Both et al. [41], a DLI of 17 mol m^{-2} d^{-1} is required to achieve adult-sized lettuce (150 g at 35 days after transplantation). These prior studies suggest that a higher DLI than the ones used in this study may have led to faster plant development, although it is important to note that light responses can vary depending on the species or cultivar.

Regardless of the plant species, our study has shown that implementing a 20-h photoperiod segmented into four lighting cycles per day (5L/1N and 5L/1LCP treatments), while maintaining the same DLI and using a slightly lower light intensity (256 or 261 µmol m^{-2} s^{-1}), resulted in a 19 to 23% increase in productivity compared to a commercially used 18-h photoperiod (control treatment of 18L/6N) with a PPFD of 289 µmol m^{-2} s^{-1}. Similarly, Kelly et al. [42] reported that a longer photoperiod and a lower PPFD (24 h at 180 µmol m^{-2} s^{-1}) increased the fresh and dry biomass of lettuce (DLI of 15 mol m^{-2} d^{-1}). Both et al. [41] also demonstrated that using lower PPFD with a longer photoperiod resulted in a similar dry weight compared to a higher PPFD over a shorter photoperiod. This effect is reflected in the light use efficiency parameter, as the fresh and dry weight-based photon yields were higher under the 5L/1N and 5L/1LCP treatments compared to 6L/6LCP and 18L/6N. Furthermore, Hiroki et al. [10] demonstrated that production efficiency (fresh weight divided by electricity consumption) is more favorable at a PPFD of 110 µmol m^{-2} s^{-1} in comparison to 170 µmol m^{-2} s^{-1}. These findings highlight the potential benefits of optimizing the photoperiod and light intensity to enhance productivity and light use efficiency in controlled environment agriculture.

4.3. Effect of Replacing the Dark Phase with an LCP Phase

The use of a 6-h lighting phase under a high PPFD of 418 µmol m^{-2} s^{-1}, combined with a 6-h "maintenance" phase under LCP of 20 µmol m^{-2} s^{-1}, resulted in yields comparable to those obtained under the control treatment (18L/6N) with a lower PPDF of 289 µmol m^{-2} s^{-1}. Nevertheless, there was no discernible benefit observed when replacing the dark period with LCP when comparing 5L/1LCP to 5L/1N. Furthermore, our lighting treatments did not have a significant impact on photosynthetic capacity, as indicated by Amax, Rd, and ϕ. On the other hand, Zhou et al. [2] reported that the photosynthetic capacity and growth rate of crops grown under a relatively long light/dark cycle (12/12 h) increased compared to the shorter light cycles (6/6 h and 3/3 h). An interesting observation

is that SLA was higher under the 6L/6LCP treatment (lower SLW), indicating thinner leaves for the same biomass. This might be a result of a shadow response triggered by 12 h of LCP during the day. Moreover, a higher SLA is typically associated with poor post-harvest quality due to increased water loss via leaf transpiration and a higher respiration rate per leaf area.

The high PI and high F_V/F_M ratio observed under the 6L/6LCP treatment for some species (spinach, lettuce cv Garrison and cv Blade, and mustard cv Scarlet Frills) suggest good plant vigor and tolerance to the highest PPFD used during this experiment (418 µmol m^{-2} s^{-1} compared to 256 µmol m^{-2} s^{-1}). This confirms the feasibility of automated vertical growing systems sharing high intensity LEDs, potentially reducing the cost of artificial lighting by employing medium–high-power LEDs that are more cost-effective in terms of dollars per watt (W) and reduces the overall number of growing lights required. However, it is important to note that using more powerful lamps for shorter periods may not be the most efficient approach in terms of electricity consumption [10].

Regarding the quality aspect, the nitrate concentration was higher under the 6L/6LCP treatment for spinach, basil, and mustard cv Scarlett Frills. These results align with the findings by Lillo [43], who reported that plants tend to accumulate more nitrates at low light intensities, especially during the nocturnal phase. However, the 5L/1LCP treatment, when compared to the 5L/1N treatment, lowered the nitrate concentration of Swiss chard. On the other hand, the SPAD value was higher under the 5L/1LCP treatment compared to the 5L/1N treatment for Swiss chard, chicory, pak choi, and arugula. This suggests a higher concentration of chlorophyll in these species when exposed to the 5L/1LCP lighting treatment.

4.4. Effect of Light Spectral Quality

The addition of far-red to a red and blue light treatment had a positive impact plant height of lettuce cv. Garrison, but did not significantly affect the other species. This is consistent with the findings of Ying et al. [44], who observed an increase in plant height when exposing different species of microgreens to red and blue light (as main PPFD) enriched with far-red light (RB + FR), without a significant impact on fresh and dry plant biomass. In our study, some modifications in plant morphology were observed, such as the size of the cotyledons of Swiss chard. Additionally, there was an 18% increase in plants' dry biomass with the addition of far-red (RB + FR) compared to the red and blue treatment (RB), although this difference was not statistically significant. However, using only blue and red light reduced the plant dry biomass by 20 to 31% compared to the other white spectrum treatments (p = 0.094), highlighting the importance of green and far-red light. Dry biomass is a crucial growth parameter in edible crops like lettuce because economic returns are directly related to dry weight [45]. Furthermore, previous research has shown that at certain light intensities (200 and 300 µmol m^{-2} s^{-1}), the total dry weight is highest at specific blue to red (B/R) light ratios (0.23 and 0.33, respectively) due to the high net photosynthetic rate and the amount of light received by the leaves [46]. In our study, the B/R ratios were 0.40 for CW, 0.32 for WW, 0.13 for RB, and 0.11 for RB + FR. Although not statistically significant (p = 0.094), the dry biomass of CW and WW was high but similar to RB+FR, while the B/R ratio was much lower, emphasizing the significant role of far-red light in dry weight. Furthermore, the higher productivity observed in indoor-cultivated leafy greens compared to those in the greenhouse from late March to early August could be linked to a potentially more challenging growth environment, characterized by temperature spikes and associated drier conditions during sunny days.

The important role of green light in influencing growth parameters has been explored in previous studies. For example, Kitayama et al. [47] found that in spinach stem and shoot fresh weights as well as leaf area, were highest under the RBG treatment (30:28:42, red:green:blue) compared to the other light compositions, such as 70:18:12, 57:16:27, and red and blue only (79:21). In our study, it is noted that a higher proportion of green light (39–40%) combined with a medium proportion of red light (41–44%) reduced the

water content of mizuna when compared to the other treatments with a lower green content (1–2%) and a higher red content (61–85%). However, no significant effect on the water content was observed for spinach. This suggests that the specific light composition, including the balance of green light, can have varying effects on different plant species, highlighting the importance of tailoring the lighting conditions to the specific needs of the crops being grown.

The effect of light on the anthocyanin concentration in plants can vary depending on the light intensity and spectrum. In a study by Fällström et al. [48], it was shown that high-intensity lighting or a heavy blue light spectrum can enhance the anthocyanin content in plants, resulting in a red/purple pigmentation. This effect was observed with the high light treatments at 450 μmol m^{-2} s^{-1} with a 13% blue light ratio or a heavy blue spectrum treatment with a PPFD of 223 μmol m^{-2} s^{-1} and a 35% blue light ratio. In our Photoperiod × PPFD experiment, there were no significant effects of the light treatments on the anthocyanin concentration, despite the use of high PPFD under the 6L/6LCP treatment (418 μmol m^{-2} s^{-1}). Zhang and Folta [49] demonstrated that green light has an inhibitory effect on the production of anthocyanins, especially when combined with blue light. However, in our experiments, despite the use of a relatively high proportion of green light (39–40%), along with the highest proportion of blue light (14–16%), there was no significant reduction in the concentration of anthocyanins in the leaves. The varying proportions of blue light (ranging from 7% to 16%) in our different treatments may have been insufficient to trigger a notable response in terms of anthocyanin production. Additionally, the anthocyanin concentration varied among the 12 leafy green species, with some having very low levels naturally. This variability among species may help explain why our lighting treatments did not have a discernible impact on the anthocyanin concentration.

5. Conclusions

Segmenting and lengthening the photoperiod improved nearly all the agronomic parameters studied for the 12 organically grown leafy green species without any detrimental side effects, even under a wide range of PPFD levels (from 256 to 418 μmol m^{-2} s^{-1}). Shorter lighting periods with medium PPFD (5L/1N and 5L/1LCP) increased SFW, SDW, and height, positively influencing the leaf thickness and potentially the shelf life due to the increased SLW and reduced water content. This resulted in a better photon yield when considering fresh and dry weight-based measurements. Extending the photoperiod by 2 h per day (20 h instead of the control 18 h) and simultaneously reducing PPFD by 10% or 11% (5L/1N and 5L/1LCP; 261 and 256 μmol m^{-2} s^{-1}) compared to the control (18 h/6N; 289 μmol m^{-2} s^{-1}) or by around 38% compared to 6L/6LCP (418 μmol m^{-2} s^{-1}), increased on average plant productivity by 21% and 12%, respectively. However, the economic trade-offs associated with extending the light phase by 2 h, when compared to the gains achieved through lower intensity and increased efficiency, will require further investigation within a commercial context.

In our study, we found that green light did not have any negative effects on the plant quality. In fact, green and far-red light tended to increase the dry biomass of plants, which is an important parameter for the quality of leafy green species. However, it is worth noting that the impact of these lighting conditions on various growth and quality parameters can vary depending on the specific plant species. On the other hand, when comparing the indoor cultivation of leafy greens to greenhouse cultivation, we observed higher productivity among the indoor plants from late March to early August, with no significant differences in terms of quality attributes. To validate the findings of this study across a broader range of plant species and to evaluate their economic implications, further complementary research and economic studies would be necessary.

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