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Combined Effects of Different LED Light Recipes and Slow-Release Fertilizers on Baby Leaf Lettuce Growth for Vertical Farming: Modeling through DoE

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Abstract: The modern agriculture system based on open-field crops requires a lot of energy and resources in terms of soil, water, and chemicals. Vertical farming (VF) systems could be a viable alternative for some types of cultivation that are receiving interest thanks to their high modularity, optimized water and nutrients use, and LEDs employment as an energy-efficient light source. However, VF design and installation are expensive and require well-tailored optimization depending on the specific crop to increase its competitiveness. This work analyzed the effects of different combinations of NPK (nitrogen-phosphorus-potassium) slow-release fertilizers and LED-based light recipes on the growth of baby leaf lettuce (*Lactuca sativa* L.), taking advantage of the Design of Experiments (DoE) methodology. The type of slow-release fertilizer, its quantity measured as the number of aggregates from 0 to 6, and the type of light recipe were considered as input factors, and their possible influence on the growth of lettuce (in terms of morphological parameters) in a controlled indoor farming system was measured. Results suggest that using higher fertilizer inputs equal to six aggregates leads to an increase of average leaf area equal to 46% (from 13.00 cm^2 to 19.00 cm^2), while the fresh weight of lettuce increases by 65% (from 1.79 g to 2.96 g). However, the height of plants also depends on the combination of the light recipes. In particular, the separate coupling of higher inputs of two fertilizers and light recipes leads to an increase in the height of lettuce equal to 33% (from 6.00 cm to 8.00 cm).

Keywords: lettuce; light emitting diode; Design of Experiments; vertical farming; slow-release fertilizers; NPK; mathematical models; indoor farming

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1. Introduction

Agriculture is currently facing a major problem with sustainability. The traditional agricultural system requires a huge amount of land surface, water, and materials, which are necessary to supply the nutrients necessary for the growth of crops. The system will be put under further pressure by the increasing global population in the next decades [1]. Vertical farming (VF) is characterized by the indoor farming of crops in a controlled environment and with the use of artificial lightning as the only source of light [2]. VF systems have many advantages that make them attractive as an alternative to conventional agriculture or suitable for the food self-sufficiency of cities [3]. In fact, the total control of the growing substrate enhances the use of water and avoids the waste of nutrients [2,4,5], while the closed environment helps to reduce the presence of pests and the use of pesticides. Temperature and humidity control avoid problems like extreme weather conditions, e.g., droughts or frosts. Moreover, the production is stable and continuous all year, resulting in higher yields with respect to open-field agriculture [6]. Finally, the use of stacked growth layers means the efficient use of space and high scalability, which allow VF placement even in working places [7]. In fact, it has been demonstrated that the addition of a VF system in an office can reduce the CO_2 concentration by up to 34%, and the energy for mechanical ventilation by up to 58%, depending on the number of people and crop growth stage [8].

However, VF systems' implementation requires optimizing every aspect of the growth of crops. The simultaneous management of different objectives is hard to achieve, e.g., the minimization of the number of shelves used, change of shelf configurations, or the unmet amount of crop demands [9]. Despite a generally positive attitude among stakeholders towards VF, the associated amount of energy required for their management and consequent high set-up prices hinder their diffusion [10]. In fact, lightning systems could require in general a high amount of energy. The electricity needed for VF management represents 66% to 85% of the total carbon footprint, which is 2.3 to 16.7 times that of conventional open field farms, depending on the type of crops and on the extension of the open field area [11]. In addition, the trade-off between the soil area saved using VF and the soil necessary for the production of energy through renewable sources (wind, photovoltaic) is not trivial. The amount of land area saved is positive only for some crops, e.g., lettuce or wheat. Otherwise, additional amounts of land are required to produce energy to power VF systems [12]. Therefore, optimization of the artificial lightning is pivotal for the future development of VF.

In this context, light-emitting diodes (LED) have many advantages with respect to fluorescent and HPS (high-pressure sodium) lamps, since LEDs are more energy efficient and economical [13,14]. LEDs also have a longer operational lifetime (30,000–50,000 h) than fluorescent (20,000 h) or incandescent lights (1000 h), and the limited emission of radiant heat allows their placement near the plant canopy [15]. While the use of supplemental artificial lighting in greenhouses accounts for no more than 25–30% of total costs, and the use of LEDs instead of HPS lamps results in economic advantages after 7 years [15], the use of solely artificial light for crops growth poses other challenges. The conversion from LED light to plant biomass (1.2–20.1%) is more efficient than that of sunlight (0.03–0.62%), even when there is the action of other factors that could lower the efficiency to 0.1–5.7%. These factors are mainly the type of plant and the presence of air conditioning in the growth environment [16].

Another advantage of LEDs is that they have tunable wavelengths, so light recipes can be tailored according to the type of plant and its stage of growth. Every wavelength acts differently on the morphology and physiology of plants [17]. However, defining tailored light recipes for specific plant species is not so easy, according to the literature, and other works should consolidate the knowledge in this field. In fact, the optimal light recipe depends not only on the plant species, but also on other factors like the growth stages or the objectives of the growth, e.g., flowering, vegetative growth, fruit, and even postharvest quality management [14,18]. In addition, environmental variables, such as humidity and temperature, must be taken into strong consideration.

Lettuce is ideal for growth in indoor controlled environments thanks to its limited height and short life cycle [19]. There are different studies concerning the effects of light wavelengths on lettuce growth. Blue and red wavelengths are the most common, though light recipes with different blue/red ratios were considered in different studies [20–23]. Far-red light is beneficial for lettuce when added to red and blue light [24], as it enhances morphological parameters, e.g., fresh weight, dry weight, and leaf area, but also lowers phytochemical concentrations [25]. However, far-red radiation action also depends on the proportion of other wavelengths [26], and its beneficial effect could be stronger if far-red is given alone in the last part of the photoperiod [27]. The effect of green light on lettuce is still not totally clear. According to different studies, its effect seems to be beneficial when added in a limited percentage (10%) to red and blue light, as it enhances the biomass accumulation in lettuce [28,29], negative if coupled with low blue radiation, and ineffective when coupled with high intensity of blue radiation [30]. However, green light may be useful with plants with a multilayer canopy, where blue and red lights do not penetrate the surface of the canopy, while green light would go further into the deeper layers of leaves, enhancing their photosynthesis [29,31].

Many factors are involved in the management of VF systems, e.g., the species and cultivar of the crop, the light intensity and wavelength composition, the photoperiod, but also environmental factors and the way nutrients are given to plants [32,33]. Therefore, an optimized and all-embracing management of VF systems must consider the interactions of these factors. This paper attempted to respond to this need, using the Design of Experiments (DoE) methodology. DoE, through a multivariate analysis of variance (ANOVA), is extremely useful for analyzing and modeling the interactions among different variables to build statistical reliable models, as already reported in previous studies [34–36]. As an innovative part of previous literature, in this work, the growth of baby leaf lettuce (*Lactuca sativa* L., cultivar Chiara) in an indoor controlled environment was analyzed considering two different light recipes and two categories of slow-release fertilizers in different quantities. Thereafter, both different types of LED lights and fertilizers have been employed as independent variables of the Design of Experiment approach in order to identify and calculate their effect on the response variables. In fact, it is well known that all these parameters can affect plant growth, but a robust statistical approach is almost never applied to perform a quantitative estimation. In addition, the slow-release fertilizers employed in this study have a core-shell structure, where the core is made of porous inorganic material and the shell is made of an organic coating, formulated from waste and by-products compounds, therefore sustainable for the environment. The nutraceutical and morphological properties of lettuce plants were analyzed and mathematically modeled with DoE to understand both the effects of single factors and their interactions. Finally, mathematical models were employed to define a specific optimized condition for the growth of lettuce in a controlled indoor environment, optimizing the use of resources needed specifically for this crop.

2. Materials and Methods

2.1. Test Materials and Growth Conditions

The aims of this work included the validation of two slow-release fertilizers which were used in growth tests with lettuce. These two fertilizers are characterized by a core-shell structure, and every aggregate weighs about 1 g. The core of both is made of a porous inorganic material that has a constant formulation shown in Table 1. Among the materials shown in Table 1, redclay obtained from northern Italy (Modena, Italy) and pumice (Europomice s.r.l., Milano, Italy) scraps (a material hard to trade due to too low particle diameter) were used as matrix materials [37,38]. Spent coffee grounds employed for this investigation were a post-consumer by-product obtained from a local coffee bar (Modena, Italy), and they were included into the core formulation as a poring agent. To enrich the cores with nutrients, animal bone meal ash, as a by-product of meat processing, and potassium carbonate (K_2CO_3, ACS reagent, $\geq$99.0%, Sigma Aldrich, Merck, Darmstadt Germany) were respectively used as sources of phosphorus (P_2O_5 content = 41 wt%) and potassium (K_2O content = 68 wt%).

Table 1. Core formulation (wt%) of the slow-release fertilizers.

Material	wt%	Function
Red clay	28.3	matrix material
Pumice scraps	41.7	matrix material
Spent coffee grounds	10.0	poring agent
Animal bone meal ash	14.0	source of phosphorus
K_2CO_3	6.0	source of potassium

It has to be specified that the two slow-release fertilizers obtained at the end of the manufacturing process differ only in terms of the method for adding nutrients into the matrix. The first type of fertilizer, called APV50, is enriched in nutrients through a fertilizer glass based on pumice scraps, animal bone meal ash, and potassium carbonate. In the second type, called APNUT, the meal ash and the potassium carbonate were simply mixed with the other compounds as they were. A detailed analysis of the two cores' properties and production processes are published elsewhere [39]. However, the main properties of the two cores are shown in Table 2.

Table 2. Properties of the two cores of the slow-release fertilizers [39].

Property	APV50	APNUT
Bulk density (g/cm^3)	1.260	1.146
True density (g/cm^3)	2.6523	2.6497
Total porosity (%)	51.88	56.14
pH	6.60	7.38
Electrical conductivity (dS/m)	0.268	0.220
Water absorption after 24 h (%)	22.00	30.73

The shell of the fertilizers was made of an organic coating realized using water (11 wt%) and biomass from black soldier fly larvae (BSFL) (89 wt%) reared on a substrate of vegetable waste. BSFL were used as the source of nitrogen, after the removal of the fat fraction, with the double aim of increasing the nitrogen content and enhancing the workability of the material. The coating formulation was defined through an optimization process based on the DoE methodology in a previous study and the content of nitrogen estimated was 7% [40].

Baby leaf lettuce (*Lactuca sativa* L.) type cultivar "Chiara" (ISI Sementi S.p.A., Fidenza, Italy) seeds were used for the growth tests. The growing substrate was a mix with a 3:1 ratio, respectively, of agriperlite "Agrilit 3" (Perlite Italiana s.r.l., Corsico, Italy) and universal peat moss soil "Potgrond H" (UNICO, AL.FE s.r.l., Pomponesco MN, Italy). The agriperlite had a density equal to 90 kg/m^3 and a particle diameter range between 2 mm and 5.6 mm. The peat soil contained 23% of organic carbon, 0.4% of organic nitrogen, and 46% of organic matter. This growing substrate, without the addition of fertilizing aggregates, was also used as a control during the growth tests. Five lettuce seeds were placed on the top of plastic pots. Each pot had a volume of 500 cm^3 that was totally filled with the growing substrate and 50 mL of water for every pot was given at the moment of sowing and then every 3 days until the end of the test, which lasted for 28 days. The tests were carried out in a growth chamber with constant temperature (24 ± 1.5 °C) and relative humidity (64–75%) conditions. Light conditions are described in Section 2.2.

2.2. Lighting Conditions and Lighting Recipes

Lettuce plants were isolated from external light sources. The only light source was a PHYTOFY® RL tunable LED panel (OSRAM GmbH, Munich, Germany), designed for indoor horticulture applications. The pots were placed under the LED module at a constant distance of 55 cm and were switched places every 3 days to compensate for possible inhomogeneous distribution of light intensity by the LED module. Some lighting conditions were common to all the growth tests. A constant photoperiod equal to a 16 h day^{-1} was applied, with light supplied continuously from 00.00 to 16.00 without interruptions. The Photosynthetic Photon Flux Density (PPFD) was equal to 150 µmol m^{-2} s^{-1}, while the daily light integral (DLI) was equal to 8.64 µmol m^{-2} d^{-1}. Two different light recipes were tested for the growth of lettuce, named LED-1 and LED-2. Their spectral composition, expressed as PPFD for the different wavelengths, is shown in Table 2, and is similar to the information given in previous work [41]. Figures 1 and 2 give a graphic representation of the light recipes, obtained from the data of the software supplied by the producer with the LED panel. Figure 1 shows the timespans of different spectra used in LED-1 and LED-2. Figure 2 instead shows the different spectra. LED-1 is made only of one spectrum with blue and hyper-red in a 50/50 ratio (Table 3, Figure 2a). This light recipe was chosen because it is more conventional and is more able to guarantee the stable growth of lettuce plants. In fact, the two hyper-red and blue wavelengths are often used for the indoor growth of plants [20–23]. The LED-2 recipe was designed with a triple aim, namely to promote the growth of lettuce through the alternation of hyper-red and blue, but also with the aim of trying the effect of other wavelengths, and finally to promote the germination of lettuce seeds using only hyper-red and far-red wavelengths. LED-2 is made up of three different spectra (Figure 2b–d). Spectrum LED-2a (Figure 2b) was used during the first 7 days of the growth test to promote lettuce germination (Figure 1b). Hyper-red and far-red wavelengths promote germination of other lettuce cultivars [42,43] and in LED-2a the hyper-red/far-red ratio was equal to 2 to avoid negative responses by seeds, such as stem elongation or leaf hyponasty, which are typical of the shade avoidance syndrome (SAS) [44]. In the last 21 days, the photoperiod was divided into two parts (Figure 1b). LED-2b spectrum (Figure 2c) was used during the first 8 h of the photoperiod, and spectrum LED-2c (Figure 2d) for the following 8 h. LED-2b and LED-2c spectra differ only in terms of the alternance of blue and hyper red wavelengths (Table 3). This alternance was set to promote the vegetative growth of lettuce plants after the germination phase, as suggested by [22,23]. The choice of using far-red and green in the LED-2 recipe was determined by their additional effect, which is considered positive for the growth of lettuce when these wavelengths are added in limited percentages to blue and red wavelengths.

Table 3. Spectral composition of the light recipes used during growth tests with lettuce.

	Wavelength (µmol m^{-2} s^{-1})				
Name	**Blue (450 nm)**	**Green (521 nm)**	**Hyper Red (660 nm)**	**Far Red (730 nm)**	**White (2700 K)**
LED-1	75		75		
LED-2a			100	50	
LED-2b	88	21		11	29
LED-2c		21	88	11	29

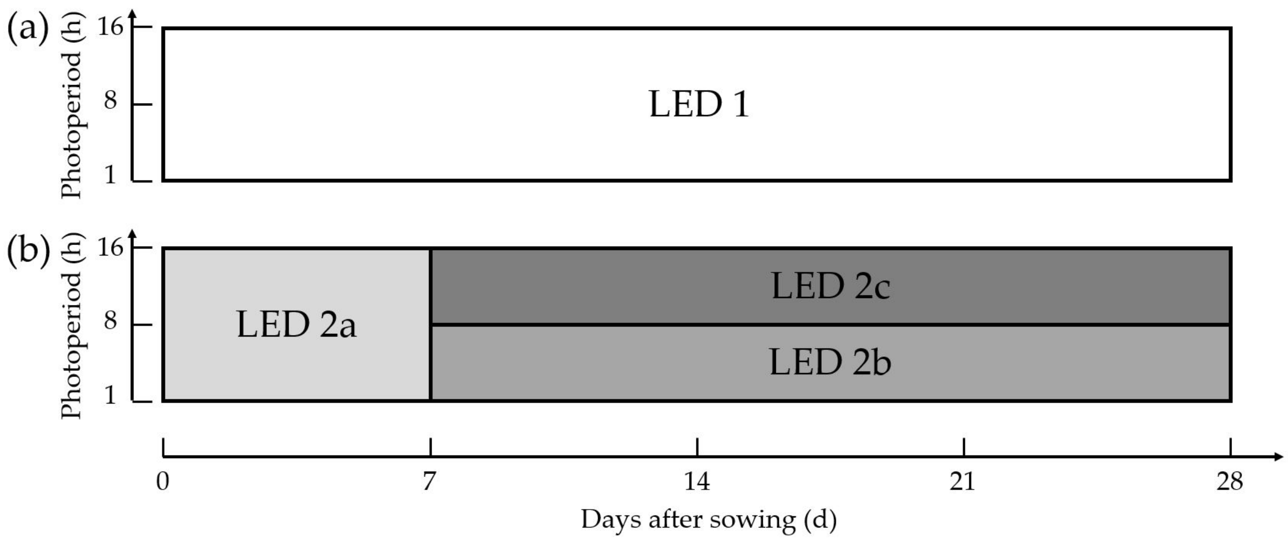

Figure 1. Time distribution of the light recipes (**a**) LED-1 and (**b**) LED-2.

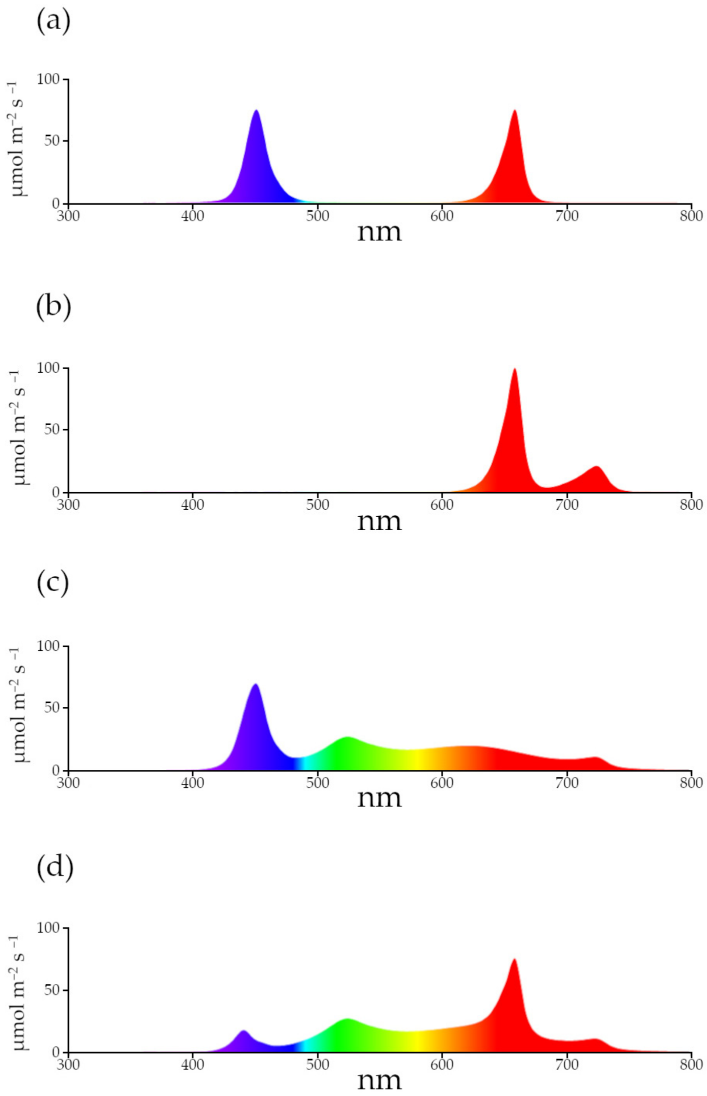

Figure 2. Spectra of light recipes used during growth tests. (**a**) LED-1, (**b**) LED-2a, (**c**) LED-2b, (**d**) LED-2c.

2.3. Experimental Plan of the Growth Tests

The DoE approach allows the minimization of experiments while considering the role of both single variables and their synergic effects due to interactions involved in the study. Design of the experimental plan, analysis of measured data, and modelization of response variables were performed using the Design Expert software (version 13.0, developer: State Ease). The definition of the experimental plan was performed considering 3 factors as independent variables. As summarized in Table 4, the factors were (a) the type of light recipe (LED), (b) the type of fertilizer (Core), and (c) the quantity of fertilizer used (Aggregates Number). Table 4 also shows the number and definition of each level for every factor. In addition, to be sure to keep constant all the other experimental conditions to perform the test, the time of the growth test execution was considered as a block, with the aim of verifying whether different days of execution of the tests would lead to significant differences, as we were investigating biological systems.

Table 4. Independent variables used for experimental plan design and statistical analysis.

Factor	Type	Number of Levels	Values
LED	Categoric/Nominal	2	LED 1 LED 2
Core	Categoric/Nominal	2	APV50 APNUT
Aggregates Number	Numeric/Discrete	3	0 3 6

Considering the independent variables and their levels, the software defined the experimental plan shown in Table 5. The number of trials allowed a reliable statistical analysis and the definition of the mathematical models of the output variables. As shown in Table 5, the software required a total of 40 experimental runs divided into 4 blocks including repetition, where every run was equal to a single pot and every block corresponded to a different time period employed for the growth test.

Table 5. Experimental plan considered for the analysis of results through DoE approach.

Run	Factor 1	Factor 2	Factor 3	Block
	Core	**Aggregates Number**	**LED**	
1	APV50	3	LED 1	1
2	APV50	6	LED 1	
3	APV50	3	LED 1	
4	APV50	6	LED 1	
5	APnut	6	LED 1	
6	APnut	3	LED 1	
7	APnut	6	LED 1	
8	APnut	3	LED 1	
9	APnut	0	LED 1	
10	APV50	0	LED 1	
11	APnut	6	LED 2	2
12	APnut	3	LED 2	
13	APV50	6	LED 2	
14	APV50	3	LED 2	
15	APnut	3	LED 2	
16	APV50	6	LED 2	
17	APnut	6	LED 2	
18	APV50	3	LED 2	
19	APnut	0	LED 2	
20	APV50	0	LED 2	
21	APV50	3	LED 1	3
22	APV50	6	LED 1	
23	APV50	3	LED 1	
24	APV50	6	LED 1	
25	APnut	6	LED 1	
26	APnut	3	LED 1	
27	APnut	6	LED 1	
28	APnut	3	LED 1	
29	APnut	0	LED 1	
30	APV50	0	LED 1	

Table 5. *Cont.*

Run	Factor 1	Factor 2	Factor 3	Block
	Core	Aggregates Number	LED	
31	APnut	6	LED 2	4
32	APnut	3	LED 2	
33	APV50	6	LED 2	
34	APV50	3	LED 2	
35	APnut	3	LED 2	
36	APV50	6	LED 2	
37	APnut	6	LED 2	
38	APV50	3	LED 2	
39	APnut	0	LED 2	
40	APV50	0	LED 2	

2.4. Characterization of Lettuce Plants

All the growth experiments followed the same timeline, with the end of every test 28 days after sowing. During every test, the number of germinated plants per pot was counted 3 days after sowing (NoP3) and 7 days after sowing (NoP7) to verify possible differences of light recipes on the germination of lettuce seeds. All the other measurements were performed at the end of growth tests, therefore on 28 day-old lettuce plants. At the end of every growth test, lettuce plants were taken and characterized singularly. Morphological properties were measured with a systematic approach, described as follows. Average fresh weight (FW) and root total dry weight (RDW) were measured using a laboratory scale (Kern analytical scale, readability 0.0001 g). RDW was measured after drying at 65 °C for 48 h using a drying oven (Argo-Lab, TCN 50). Average plant height (Height) was measured using a digital caliper with resolution 0.01 mm and accuracy ± 0.02 mm (Borletti CDJB15-20). The average leaf area (LA) refers to the area of the single leaf and not to the total area of leaves of a single plant. It was measured for the three greatest leaves of the single plant, for two plants in each pot. Images of leaves were taken using a Canon EOS 1100D digital camera always placed in the same position. Images were elaborated using ImageJ software (Bethesda, MD, USA, version 1.52). Certified methods were used to perform the chemical analysis regarding organic carbon (C), nitrates (NO_3), aluminum (Al), silicon (Si), and the solid residue at 105 °C (SR). C was quantified using the ANPA 2001 methodology [45], NO_3 according to [46], Al according to [47], Si according to [47], and SR according to [48]. Two leaf samples were taken from each pot for the quantification of chlorophyll a (Ch_a), chlorophyll b (Ch_b), and carotenoids (Car). The samples were treated according to Lichtenthaler's method and a UV-vis spectrophotometer (Jasco, V730) was used to measure the absorbance [49].

2.5. Statistical Analysis

The properties shown in Table 6 were considered for the ANOVA analysis and consequent modeling of the data capable of defining the lettuce growth through single and interaction effects of the different light conditions and fertilizer addition. A necessary condition for ANOVA is the orthogonality of the factors that must also be normally distributed [50]. Thereafter, by employing the Fisher F-test, it is possible to estimate the variance homogeneity and the corresponding p-value for each term (in single and interaction) of the model. In addition, with the same test it is also possible to evaluate the residual not explained by the model and related to the control and fixed variables [50]. As a threshold for significance, a p-value lower than 0.05 was considered in this study, and the R^2 and Pred-R^2 values were considered to evaluate the quality of the model fit [44]. After the calculation of all the models, a desirability function was calculated to provide the most desirable artificial light and fertilizer condition, taking into account the average of all the responses analyzed according to their specific goals and importance (Table 7) [50].

Table 6. Response variables used to characterize grown lettuce plants.

Response Variable	Acronym	Unit of Measurement
Average fresh weight	FW	g
Root total dry weight	RDW	g
Average plant height	Height	cm
Number of plants after 3 days	NoP3	/
Number of plants after 7 days	NoP7	/
Average leaf area	LA	cm^2
Organic carbon	C	%
Nitrates	NO_3	mg kg^{-1}
Aluminum	Al	mg kg^{-1}
Silicon	Si	mg kg^{-1}
Solid residue at 105 °C	SR	%
Chlorophyll a	Ch_a	µg mg^{-1}
Chlorophyll b	Ch_b	µg mg^{-1}
Carotenoids	Car	µg mg^{-1}

Table 7. Desirability function parameters.

Response Variable	Goal	Importance [Min 1–Max 5]
Average fresh weight	to maximize	4
Root total dry weight	to maximize	5
Average plant height	to maximize	5
Number of plants after 3 days	to maximize	3
Number of plants after 7 days	to maximize	5
Average leaf area	to maximize	5
Organic carbon	to maximize	2
Nitrates	to minimize	3
Aluminum	to minimize	3
Silicon	to minimize	3
Solid residue at 105 °C	to minimize	5
Chlorophyll a	to maximize	4
Chlorophyll b	to maximize	4
Carotenoids	to maximize	4

3. Results and Discussion

The results of all the characterizations of the lettuce plants are reported in Table 8. From a first consideration of the measured data, it is possible to assess the possible benefit of the statistical approach. In fact, it must be considered that each response shows fairly good data variability among the selected range of the independent variable. This fact suggests that the selected input factors may affect the responses and thereafter a mathematical model can be drawn. Nevertheless, it is not possible to evaluate a specific trend at this point of the analysis; therefore, a statistical analysis should be performed, and a quantitative calculation of the effects of the input factors on the selected responses, also considering the block related to each different time period.

Table 8. Results of the characterization of lettuce plants.

Run	FW g	RDW g	Height cm	NoP3 /	NoP7 /	LA cm^2	C %	NO_3 mg kg^{-1}	Al mg kg^{-1}	Si mg kg^{-1}	SR %	Ch_a µg mg^{-1}	Ch_b µg mg^{-1}	Car µg mg^{-1}
1	3.10	0.3216	5.950	2	5	14.99	2.7	24.9	1.9	58.5	6.25	0.2228	220.6173	0.1806
2	3.86	0.5075	5.700	2	4	18.38	3.3	1.0	3.1	68.7	8.16	0.3593	35.6345	0.9479
3	3.99	0.3967	6.000	1	5	18.06	4.0	18.0	2.6	65.8	8.35	0.2756	334.6319	0.2647
4	4.09	0.5063	6.125	2	4	17.01	2.8	5.6	2.2	74.8	6.56	0.3276	96.1384	0.3380
5	3.97	0.3379	6.525	0	2	18.32	2.7	20.0	2.5	77.7	6.44	0.4592	131.2246	0.3902
6	3.24	0.4101	7.000	2	5	18.45	2.9	65.4	2.6	61.1	7.35	0.2581	376.8204	0.2113
7	3.38	0.3536	7.640	4	5	20.54	2.5	2.8	1.8	50.2	6.16	0.2567	364.3097	0.2004
8	2.62	0.3650	6.500	3	5	18.16	2.7	171.0	2.5	56.4	6.34	0.5162	162.6527	0.5412
9	2.29	0.3443	3.333	2	4	10.89	4.3	6.2	3.2	73.5	10.25	0.0996	335.1883	0.0668
10	1.72	0.4444	3.400	5	5	10.25	4.1	0.2	3.7	105.9	10.28	0.4118	264.1289	0.3335
11	2.49	0.3791	5.125	3	4	16.93	4.4	14.1	2.2	72.3	9.88	0.2228	220.6173	0.1806
12	3.00	0.2964	6.020	2	5	15.01	3.0	69.6	1.5	30.7	6.02	0.3593	35.6345	0.9479
13	1.76	0.3319	5.475	4	5	12.14	6.4	12.8	4.2	108.7	13.26	0.2756	334.6319	0.2647
14	2.59	0.5444	5.860	3	5	16.10	3.5	6.0	2.4	72.1	8.00	0.3276	96.1384	0.3380
15	3.34	0.5442	6.500	5	5	14.89	4.6	14.7	2.5	52.0	7.83	0.4592	131.2246	0.3902
16	2.01	0.3494	6.100	4	5	12.63	5.1	11.2	2.7	61.7	11.19	0.2581	376.8204	0.2113
17	4.38	0.3966	7.200	3	3	19.15	3.4	5.8	2.2	32.1	6.66	0.2567	364.3097	0.2004
18	2.19	0.4702	5.840	3	5	15.92	4.8	6.2	3.0	71.1	10.10	0.5162	162.6527	0.5412
19	1.79	0.3498	4.600	4	4	10.37	6.0	4.7	3.2	75.1	10.39	0.0996	335.1883	0.0668
20	1.91	0.4032	4.860	5	5	10.05	4.2	0.7	2.5	40.0	9.87	0.4118	264.1289	0.3335
21	1.82	0.4290	7.135	5	5	16.69	3.0	3.1	1.2	68.8	7.00	0.2091	208.7652	0.2126
22	2.15	0.3052	9.096	3	5	24.06	3.0	4.7	3.0	98.2	7.92	0.3251	140.3424	0.2809
23	2.54	0.3887	8.174	4	4	19.09	3.3	4.5	0.9	72.5	7.20	0.2106	177.9564	0.2193
24	1.89	0.3491	8.294	3	5	17.33	3.4	4.5	1.1	65.8	7.40	0.1909	222.7314	0.1657
25	1.77	0.2272	8.100	2	5	17.73	2.5	6.5	1.5	90.5	6.90	0.2913	162.4694	0.2591
26	2.11	0.4007	8.338	3	5	21.29	3.5	6.7	0.9	83.3	8.30	0.4766	84.9374	0.3755
27	1.68	0.3611	7.038	3	5	14.05	3.3	7.1	1.0	81.9	7.50	0.4916	147.4538	0.3633
28	1.94	0.3119	8.642	3	5	17.49	3.3	5.7	1.3	67.3	8.30	0.2328	176.9615	0.1688
29	1.39	0.1825	7.258	4	5	13.16	2.9	10.0	1.2	62.6	7.60	0.2269	183.7447	0.1767
30	1.43	0.2041	6.577	3	5	12.38	3.6	10.2	2.1	50.7	6.60	0.2661	201.8779	0.2070
31	2.69	0.1905	9.394	3	5	23.80	2.6	4.1	1.2	29.8	5.20	0.2091	208.7652	0.2126
32	2.88	0.2271	9.156	3	5	23.86	3.1	3.0	1.2	48.6	6.30	0.3251	140.3424	0.2809
33	3.32	0.2820	9.573	1	4	25.89	2.9	4.9	1.3	59.3	7.00	0.2106	177.9564	0.2193
34	2.80	0.3216	9.090	2	4	22.20	2.9	2.9	1.0	46.4	6.10	0.1909	222.7314	0.1657
35	2.89	0.4588	7.762	5	4	25.70	2.5	2.7	0.9	54.2	5.98	0.2913	162.4694	0.2591
36	4.53	0.5297	9.484	5	5	26.42	2.5	2.2	0.8	42.8	5.60	0.4766	84.9374	0.3755
37	2.75	0.2361	8.050	3	4	23.55	2.7	139.0	1.3	51.3	6.40	0.4916	147.4538	0.3633
38	3.18	0.4737	8.996	4	5	23.72	2.8	2.3	1.0	47.7	6.40	0.2328	176.9615	0.1688
39	2.15	0.9557	5.425	5	5	17.25	3.3	8.6	0.6	51.2	7.50	0.2269	183.7447	0.1767
40	1.89	0.3456	6.822	4	5	17.64	3.3	5.7	0.9	38.3	7.30	0.2661	201.8779	0.2070

The statistical analysis highlighted that there is no difference among the same runs performed in different blocks, shown in Table 5. This confirms the repeatability of the growth tests characterized by the same light recipe and the comparability of their results. The ANOVA results (Table 9) showed that input variables have a significant influence only on five response variables out of the fourteen considered. The values of R^2 and predicted R^2 (Pred-R^2) are reported in Table 9, together with the coefficients that quantify the influence of every input variable on the single response variable. The morphologic properties, namely FW, Height, and LA, have higher R^2 and Pred-R^2 with respect to the chemical properties (C and SR). It must be stressed that the Pred-R^2 related to C and SR is insignificant and, therefore, these two models cannot be employed for predictive purposes but only for the description of responses considering the already collected data.

Table 9. ANOVA results for the significant response variables.

Response *	F-Value	R^2	Pred-R^2	Coefficients Name and *p*-Value	Value
FW (g) **	60.76	0.64	0.51	Aggregates Number (<0.0001)	−0.1080
Height (cm)	16.92	0.62	0.38	Core (0.0012) Aggregates Number (<0.0001) LED-Aggregates Number (0.0056)	+0.0287 +1.0700 +0.3075
LA (cm^2)	48.80	0.59	0.44	Aggregates Number (<0.0001)	+3.3200
C (%)	9.84	0.38	0.01	Aggregates Number (0.0015) LED-Aggregates Number (0.0024)	−0.8182 +0.6038
SR (%)	11.11	0.41	0.08	Aggregates Number (0.0005) LED-Aggregates Number (0.0019)	+0.0149 −0.0091

* all the responses' residuals are not significant having *p*-value > 0.01. ** To this response a mathematical transformation (1/Y) has been applied to all the data for normalization.

Taking into account the coefficients in Table 9, the input variable that has the most diffuse effect is the Aggregates Number alone or in combination with LED. The values of these coefficients are graphically reported in Figure 3 for a better understanding. The Aggregates Number, taken alone, is also the only variable with negative values, in particular for the response variable and C (Figure 3d), meaning that increasing the number of aggregates in general could not be a viable way to promote lettuce growth, as too many nutrients could have a toxic effect on this cultivation. On the other hand, the number of aggregates positively influence the Height (Figure 3b), LA (Figure 3c), and SR (Figure 3b). According to Figure 3a,c, FW and LA are influenced only by the number of aggregates. This variable also has a positive effect when in combination with LED, indicating that the appropriate conditions of artificial light can promote a beneficial effect of the core-shell aggregate on plant growth. The synergic effect of LED and Aggregates Number is positive for Height (Figure 3b) and C (Figure 3d) and is negative for the SR (Figure 3e). Finally, it appears that the number of aggregates generally has a greater effect than that given by the LED-aggregates number. To summarize, the number of aggregates itself promotes higher plants with larger leaves and fresh weight, but also with a lower carbon content, suggesting that the higher amount of nutrients is not totally favorable to the development of new organic matter, even if it promotes other morphologic characteristics. The synergy of the number of aggregates with LED promotes higher plants with a greater content of carbon, indicating that the appropriate combination of nutrient amount and light condition can influence both the morphology and nutraceutical characteristics of plants. This combination is also useful for tailoring the characteristics of plants at the moment of harvest, depending on the grower's needs.

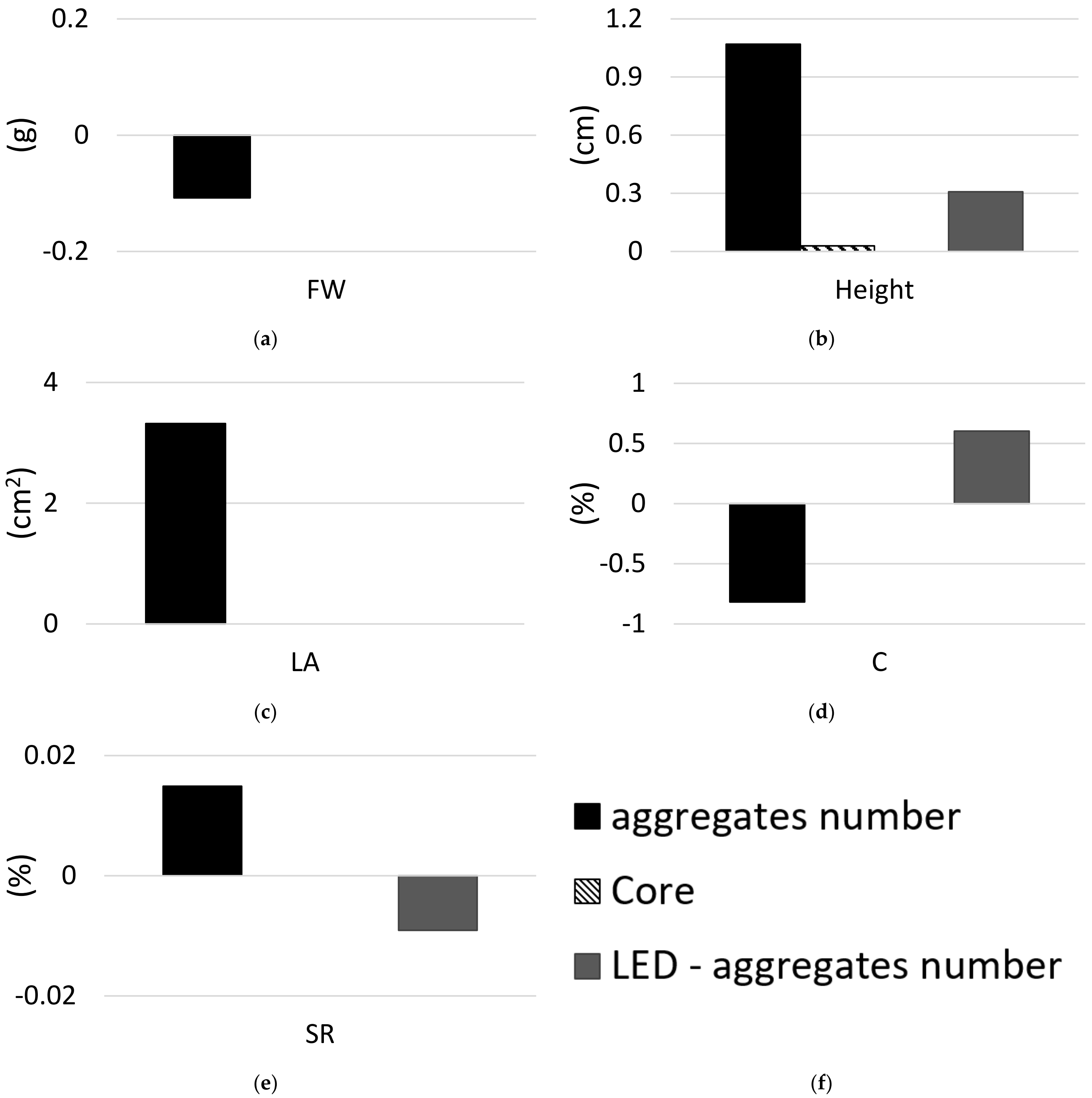

Figure 3. ANOVA coefficients for the influence of input variables on response variable, namely (**a**) fresh weight, (**b**) plant height, (**c**) leaf area, (**d**) organic carbon content, and (**e**) solid residue. (**f**) legend of input variables.

Figures 4 and 5 show the graphical expression of the mathematical models calculated for each of the five significant responses as indicated in Table 9. Figure 6 shows qualitatively the morphology of plants grown with a different number of aggregates. The model for FW (Figure 4a) suggests a non-linear increase of that property when the number of aggregates increases. More precisely, the average fresh weight of lettuce is equal to 1.79 g when zero aggregates are used and is equal to 2.96 g when six aggregates are used, with an increase of FW equal to 65%. This indicates a general positive correlation between the amount of fertilizer added and the FW developed by the plants, promoting the hypothesis that both fertilizers have a good action on the growth of lettuce. This is a positive effect, since lettuce plants with higher fresh mass means a higher yield. In this case, the type of fertilizer does not have an effect on the accumulation of a fresh mass of lettuce. This means that the presence of the fertilizer glass does not affect the yield of lettuce, so the use of the fertilizer glass is not strictly recommended for the increase of fresh lettuce mass. According to Figure 4a, the best quantity of fertilizer for lettuce is the maximum considered in this study, namely six aggregates. The model suggests that the use of greater quantities of fertilizer could further increase FW without problems, since no signs of phytotoxicity were detected on the plants. Also, the use of greater quantities of fertilizer could lead to an increase of lettuce yield without any complication, since the slow release should avoid the excessive release of nutrients into the growth substrate. The other two responses related to morphologic properties show a linear behavior. In fact, LA (Figure 4b) shows a stable linear increase with a higher number of aggregates, which is again the only factor that is statistically reliable for this response. In this case, the average leaf area of the lettuce is equal to 13.00 cm^2 when no aggregates are used and is equal to 19.00 cm^2 when six aggregates are used, with a percentage increase of LA equal to 46%. This result highlights again the positive effect of both fertilizers on the growth of lettuce, since lettuce plants with broader leaves are an indicator of greater development. The increase of both LA and FW is an indicator of the health of the lettuce, since the plants obtained using six aggregates (Figure 6c,d) show a higher yield and development compared to plants obtained using no aggregates (Figure 4a,b). A different behavior is shown by Height that is highly dependent on the type of aggregates (in single) and on the type of light recipe (in interaction). The Height increases linearly with the number of aggregates, and this happens for both APV50 (Figure 4c) and APnut (Figure 4d), as the height of plants with six aggregates (red dashed lines) is always higher than the height obtained using no aggregates (black dashed lines). In particular, when APV50 fertilizer is coupled with the LED-2 recipe (Figure 4c), the use of zero aggregates corresponds to a height of 6.00 cm, while the use of six aggregates leads to a height of 8.00 cm, with a percentage increase equal to 33%. The same numbers are obtained using APNUT fertilizer (Figure 4d) but, in this case, the maximum height is reached when the LED-1 light recipe is used. In fact, the comparison in Figure 4c,d shows that the two combinations, LED-2/APV50 and LED-1/APNUT, of the light recipes and fertilizer type, each with six aggregates, allow lettuce to reach its maximum height, equal to 8.00 cm. On the contrary, the model suggests that the two combinations, LED-1/APV50 and LED-2/APnut, when applied with six aggregates result in a lower height of plants, equal in both cases to 7.00 cm. These findings may indicate that the two fertilizers have a different release pattern of nutrients, in particular for nitrogen and phosphorus, as previously highlighted in [39]. Each of the two fertilizers combines better with one of the two light recipes, suggesting that each recipe promotes the exploitation of different nutrients, as also suggested by other authors [51,52].

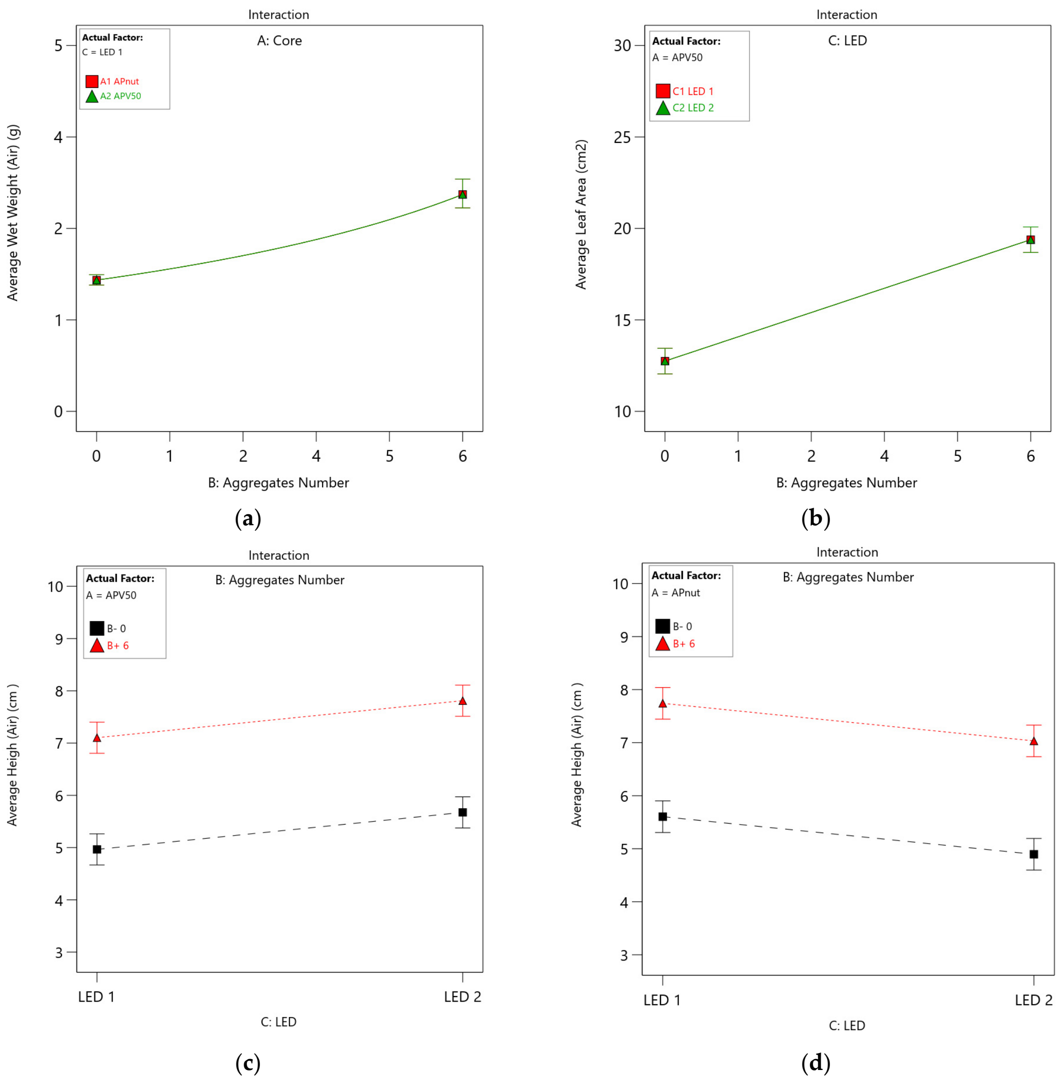

Figure 4. Interactions among the input variables for the response variables (**a**) FW, (**b**) LA, (**c**) Height considering the APV50 core, (**d**) Height considering the APnut core.

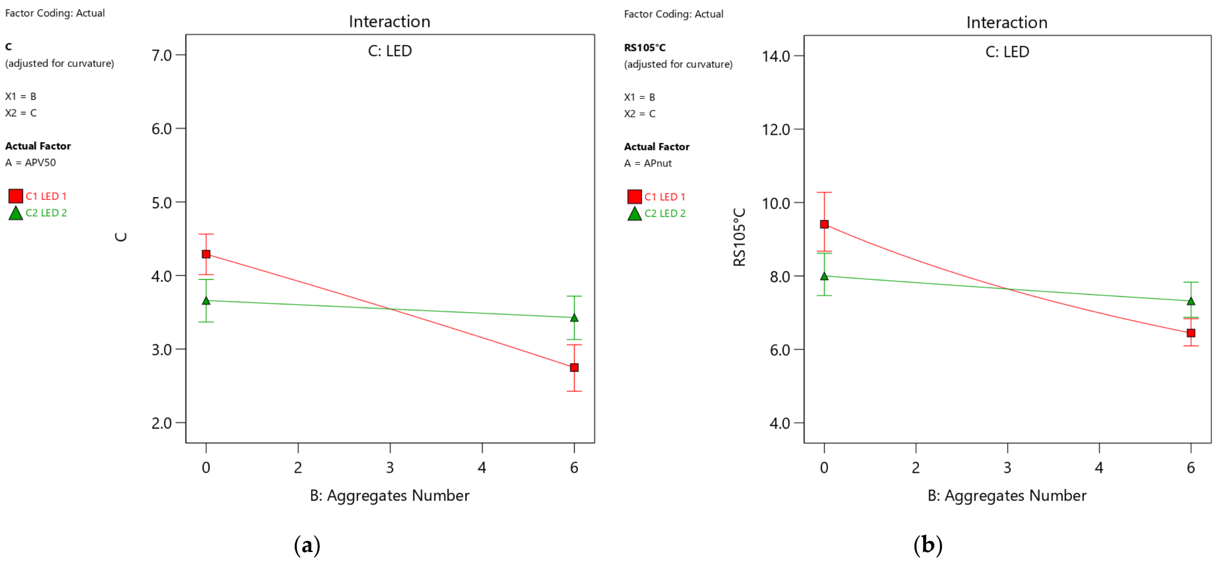

Figure 5. Interactions among the input variables for the response variable (**a**) C, (**b**) SR.

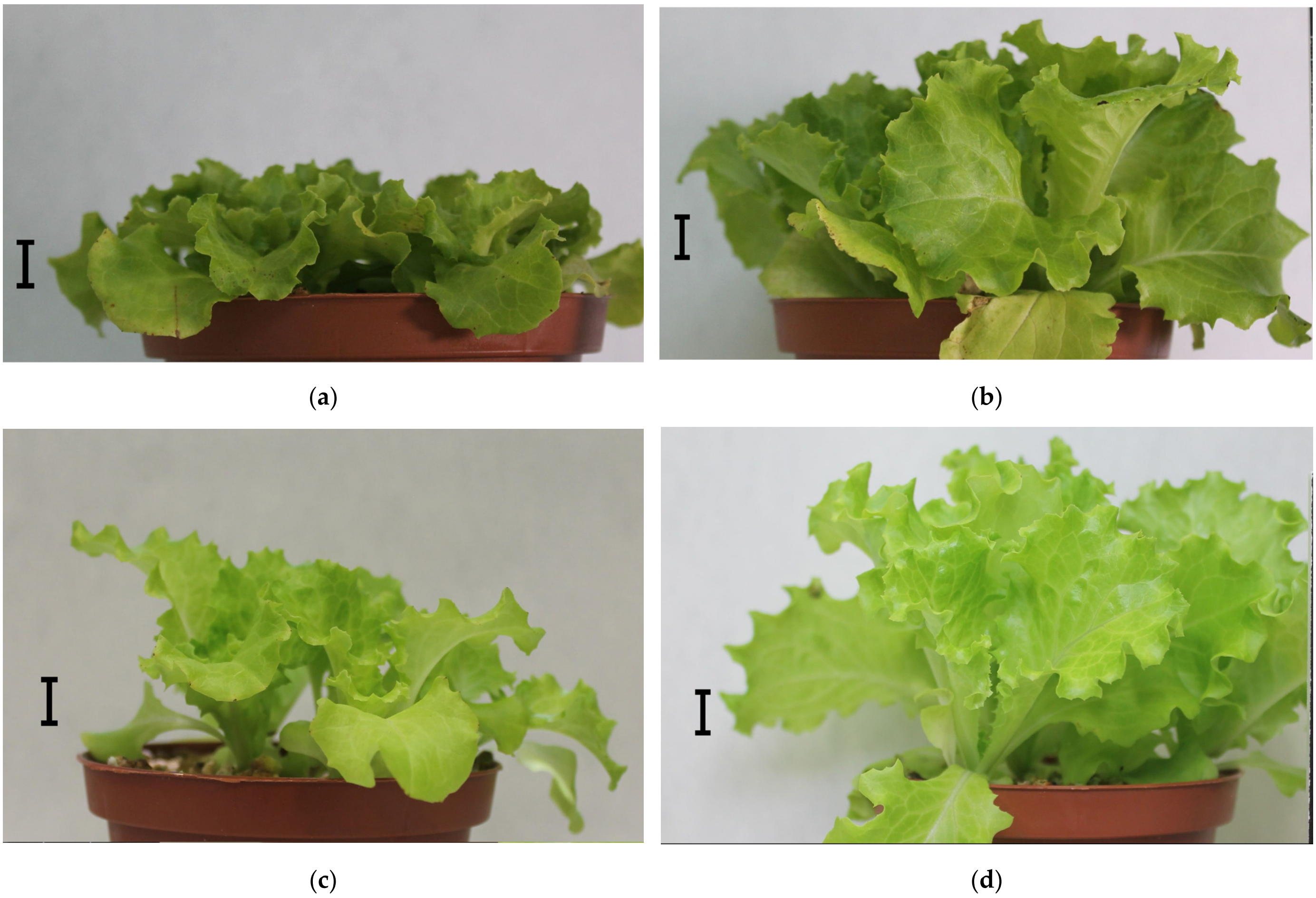

Figure 6. Examples of plant morphology occurring with (**a**) 0 aggregates LED-1, (**b**) 6 aggregates LED-1, (**c**) 0 aggregates LED-2, and (**d**) 6 aggregates LED-2. The black reference bar corresponds to 10 mm.

The response variable C represents the content of organic carbon of plants, which is also an indicator of the content of soluble sugar in the plants. As shown in Figure 5a, C is mainly influenced by two factors according to Table 8, namely the Aggregates Number and the light recipe. However, it is worth noting that the LED-2 recipe guarantees a stable content of C near 3.7%, independently from the number of aggregates, while the LED-1 recipe tends to lower C with the increase of aggregate amounts. The maximum value of C is 4.5% with no aggregates, while the minimum value is 2.8% with six aggregates. The type of aggregate seems not to influence the response variable C, consistent with Table 8. The different behavior of C in LED-1 and LED-2 could be explained by considering the positive synergy among the Aggregates Number and the LED variables, which act differently according to the light recipe used. In fact, it seems that this synergy is greater for LED-2 than for LED-1. The response variable SR (Figure 5b) shows a behavior similar to that of C, since its value remains almost constant at near 7.9% with the LED-2 light recipe but decreases in a non-linear way with LED-1, from 9.5% to 6.8%. This behavior could be explained by the use of green and far-red wavelengths in the LED-2 light recipe. Green light penetrates the deeper strata of the plant canopy and allows the whole structure of the plant to perform the photosynthesis, promoting the fixation of CO_2 on the abaxial side of leaves better than blue and red wavelengths [29,31], while red and blue are intercepted by the adaxial side of leaves and the upper canopy, which is saturated [53]. The role of the green wavelength is also recognized in the increase of dried mass in lettuce plants, when green is coupled with other wavelengths and added in a limited amount [54]. Also, the far-red wavelength has a role in helping the development of the dry mass of plants when coupled with other wavelengths [55], even if at a low percentage [25]. This would be consistent with the relatively greater values of C and SR found in plants when LED-2 is used, and the number of aggregates is higher. Therefore, the model suggests that using the maximum amount of fertilizer, equal to six aggregates, is more beneficial with the LED-2 light recipe. This is because the use of other wavelengths like green or far-red enhances the CO_2 uptake and biomass development in lettuce when the plants grow in a substrate enriched with nutrients. These nutrients promote the morphologic properties of the lettuce (Figures 4 and 6b,d), allowing the plants to develop a deeper canopy, which hinders the penetration of red and blue light in the lower leaves or in the leaves' abaxial side. On the contrary, when no fertilizer is used, the morphologic development of lettuce is lower, as shown again in Figure 4 and also in Figure 6a,c, and the use of only red and blue is enough to saturate the whole canopy of lettuce plants, since there is no deeper strata. A possible way to enhance the action of green and far-red light could be to add far-red at the end of the 16 h photoperiod, as suggested in [55], to avoid the reduction of the leaves' absorbance of the green spectrum. However, C and SR have higher values when the Aggregates Number is lower and the LED-1 light recipe is used (Figure 5a,b), suggesting that lettuce plants may develop better with only blue and red wavelengths when less nutrients are available. A possible explanation for this phenomenon is that, when plants show lower FW and LA, the canopy is less developed and deep, and the role of green and far-red wavelengths is less important.

As pointed out in [56], VF systems have a high potential for the food industry but still show several uncertainties. The main problems pointed out are the choice of lighting, nutrient delivery, irrigation, and climate control systems. The system studied in this work tried to help face some of these challenges.

- Regarding lighting, this study highlighted the following benefits: the suitability of the two light recipes for the growth of lettuce and the possibility of reducing the plant canopy–LED distance, reducing the volume needed for every single plant. The high scalability of the system can facilitate the creation of bigger facilities. One of the main limiting factors in the expansion of VF concerning the use of traditional artificial light sources (e.g., HPS lamps) is that they have high costs, high energy consumption, limited lifetime and, more importantly, a fixed light spectrum (e.g., HPS lamps). All these limits are overcome by using LEDs. First, LEDs allow the obtaining of fully customizable light recipes in terms of an adjustable light spectrum and irradiance depending on both the type of plant we want to grow and the specific phase of the growth cycle. Second, they have a power consumption that is at least 50% lower than HPS lamps for a given irradiance and a lifetime that is at least three times higher with a cost that has been constantly decreasing in recent years with a confirmed trend for the next year. Of course, less power consumption to guarantee a given level of irradiance means less need for energy and, consequently, using LEDs in VF has a significant benefit in terms of sustainability and CO_2 footprint. Moreover, VF with LEDs allows the increase of the product yield per square meter of soil because it allows the exploitation of multi-plant systems. The lower space needed for the VF system also allows it to be placed in urban scenarios with lower costs. The placement of big VFs close to (or even in) urban centers means that logistics costs are also drastically reduced and, once again, this is beneficial for the environment because both the number and operation time of vehicles producing high emissions (in primis, tractors and trucks) are reduced. In addition, reduced logistics costs theoretically allow a lower final price for the product paid by the consumer.
- Regarding nutrient delivery and irrigation systems, the use of a substrate culture system based on perlite and peat, coupled with the slow-release fertilizers, solves the problem of nutrients delivery, leaving only the need for irrigation. The use of irrigation systems in VF allows for water consumption that is almost 10% of that of open field agriculture REF. A further benefit of this system is that no pest control systems are needed, and no pest problems were noticed during the growth experiments performed

in this study. Greater VF systems will probably need some pest monitoring systems but the use of pesticides is usually near to zero.
- Regarding climate control systems, the reduction of volume required for the growth of every lettuce plant when using LED instead of HPS lamps reduces the total volume of space that needs to be air conditioned.

One of the main drawbacks of this system is that it was optimized only with lettuce, while every crop needs specific tailored light conditions and nutrients supply. Other drawbacks include the greater amount of energy needed by VF systems with respect to traditional farming methods, e.g., open field or greenhouse farming. The economic profitability of this system depends on many factors. Among them are the price of energy and the market demand for the specific crop. VF systems require a higher initial economic investment, but they have been demonstrated to be more profitable compared to systems based on HPS lamps after a limited number of years [15]. The environmental sustainability of a VF system also lies in the energy mix at the base of the energy used, as demonstrated by [57].

4. Conclusions

In this work, we used the Design of Experiments methodology to combine and evaluate the effects of two types of slow-release fertilizers combined with two different light recipes on the indoor growth of baby leaf lettuce (*Lactuca sativa* L.)

To summarize, the following conclusions could be drawn from this study:

- Among the fourteen properties considered as response variables, five produced statistically validated results. Three of them are morphologic properties, namely FW, LA, and Height of plants. Two of them are chemical properties, namely C and RS.
- The quantity of fertilizer, expressed as number of aggregates and considered in three levels (zero, three, and six aggregates) is the factor that has a greater effect on lettuce growth. Use of the highest fertilizer input guarantees an increase in fresh weight (FW) of lettuce equal to 65% and an increase in average leaf area (LA) equal to 46%.
- The combined effect of both the fertilizer type and quantity with the light recipes leads to an increase in plant height (Height) equal to 33%. This value is reached using the highest amount of fertilizer and two different combinations of fertilizer type and light recipe.
- The two chemical properties of lettuce, namely organic carbon content (C) and solid residue at 105 °C (SR), depend on the amount of fertilizer and on the light recipe used. C and SR show almost constant values of 3.7% and 7.9%, respectively, when the LED-2 light recipe is used, independently from the amount of fertilizer used. However, when the LED-1 light recipe is applied, C decreases from 4.5% to 2.8% with increasing amounts of fertilizer. SR shows the same behavior, decreasing from 9.5% to 6.8%. This could be because the LED-2 light recipe may allow the better exploitation of nutrients contained in the fertilizer.
- In the future, the use of higher quantities of fertilizer could be investigated to verify additional growth benefits or the existence of a threshold beyond which there are no further effects or negative effects, e.g., phytotoxicity for seedlings. The use of more fertilizer could also lead to the use of the same growth substrate in two subsequent growth cycles, to maximize its exploitation.

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