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Basil plants grown under intermittent light stress in a small-scale indoor environment: Introducing energy demand reduction intelligent technologies

Dafni Despoina Avgoustaki, Jinyue Li, George Xydis*

Department of Business Development and Technology, Aarhus University, Birk Centerpark 15, 7400, Herning, Denmark

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ABSTRACT

Vertical farming is a novel type of farming for fresh food production in the urban environment. Vertical farms are located in indoor environments with artificial solar radiation, completely insulated and protected from outdoor environmental conditions. Since cultivated plant species need many hours of light daily to meet their growth requirements, energy costs are very high, which can be an inhibiting factor for the advancement of the technology. In this research study, we tested the growth rate of basil plants (Genovese species) under two lighting systems. At first, the plants were grown under 16 h of continuous light, and in the second lighting system, under a photoperiod of 14 h with intermittent light. The light intensity was stable in both treatments. The purpose was to determine if intermittent light exposure could reduce the energy consumption of basil grown in indoor environments sufficiently and efficiently without adversely affecting the growth rate and biomass production of the plants. The results of the study showed that the intermittent lighting system, in which light was emitted intermittently in short (10-min) light cycles, did not affect negatively the quality and quantity of basil plants. It was found that the short light intervals were not sufficient to attain the optimal photosynthetic efficiency of the cultivation, while the overall photosynthetic rate did not decrease significantly under the indoor conditions. Finally, the evaluation of the energy footprint under various light treatments can have a positive impact on the energetic, economic, business, and ecological phases of indoor food production.

1. Introduction

Vertical farming is a novel type of farming that provides the opportunity to cultivate plant species in an indoor, fully automated environment with a continuous monitoring of the growth and development status of the plants. Vertical farming has several benefits compared to outdoor farming: No crop loss due to severe weather conditions, year-round crop production, improved water-use efficiency (up to 95%), and increased crop yield (76–116%) compared to traditional farming (Srní et al., 2015). Moreover, vertical farms replace solar radiation with artificial lighting, and in urban horticulture, conventional lighting is often replaced by light-emitting diodes (LEDs), which focus on specific wavelength ranges activating the spectral response of plant growth and development. LEDs convert 45% of their power supply into visible light absorbed by plants and can be used as a light source throughout the entire production process, while the remaining heat requires efficient heat removal through efficient thermal management. Modern LEDs in horticulture are usually equipped with a heat dissipation system, which performs heat sinking using anodized aluminum extrusion, enabling them to achieve efficacies up to 3.2 μmol/J. Furthermore, based on Xydis et al. (2020), vertical farming is a farming business scenario that allows high profitability as well as multiple revenue streams and business models in combination with small-scale wind turbines.

According to previous research (Avgoustaki & Xydis, 2020), the electricity costs of a vertical farm accounts for the highest portion of the operating costs with around 25% of the production costs. More specifically, the lighting of a vertical farm accounts for 80% of the total electricity costs. As a result, many researchers are trying to optimize the light dimensions in vertical farms to reduce operating costs. This involves light duration (photoperiod), light quality (combined wavelengths in nanometers), and light quantity (light intensity known as the photosynthetic active radiation (PAR), i.e., the solar radiation with wavelengths between 400 and 700 nm). When optimized, these factors can reduce energy costs and increase crop quality and biomass. LEDs create electron movements in a semiconductor material, and thus they can reach the most crucial nm for plants’ growth and development by activating the photosynthesis process. For this reason, a typical LED for horticulture shows a peak wavelength in the absorption spectrum at 660 nm (deep red), 450 nm (deep blue), 525 nm (green), and 735 nm (far-red) (Ashdown, 2015). LEDs perform higher correlated color temperature(CCT) compared with HPS lamps as they perform higher levels of CCT that provide more power at the short wavelengths of the spectrum. Furthermore, LEDs can produce up to 134% more bright light compared to HPS lamps even in similar illumination levels (Fotios & Cheal, 2011). The primary function of LEDs is to satisfy the require-

* Corresponding author.
E-mail addresses: gxydis@gmail.com (G. Xydis)

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ments of leaf optical properties that include different chlorophyll pigments to capture the energy and initiate the photosynthesis process.

As mentioned by Touliatos, Dodd, and McAinsh (2016), photosperiodism (i.e., the duration of light that plants receive daily) can affect the productivity and quality of the canopy at different growth stages of plants (germination, vegetation, flowering, etc.). According to Sugu
maran et al. (2013), both optimal light intensity and photoperiod can increase the yield of plants. This was further elaborated by Koza (2018), who explained that after plant seeding and through their development, the demand for photosynthetic photon flux density (PPFD) can be reduced up to 50%.

The photosynthetic processes of plants have three different types of time constraints: 1) primary photochemistry, 2) electron shuttles, and 3) carbon metabolism (Yeh & Chung, 2009). According to Matthijs et al. (1996), longer dark periods tend to reduce the growth rate of plants. However, a decrease in light flux under intermittent light seems to reduce the growth rate less than in the case of a similar flux decrease under a continuous lighting system. Intermittent light is a technique that allows us to evaluate plants’ non-photochemical reactions, which are correlated with the reduction of photochemical reactions (Briggs, 1935). In other words, during intermittent light, the light is emitted intermittently in short cycle periods. The ultimate goal of using intermittent light is to design a control energy system per growth cycle caused by saturation of the photochemical reaction. Intermittent light helps define the relationship between photochemical and non-photochemical reactions of the plants, which are related to the photoperiod.

To study whether an intermittent lighting method induces stress on plants, we examine the photosynthesis rate of basil and other physiological parameters. Photosynthesis is the process by which electromagnetic radiation (light) is converted to chemical energy, using light, water, and carbon dioxide to release carbohydrates and oxygen. The process starts with the chlorophylls (the photosynthetic pigments) that absorb light energy, while air-containing oxygen and carbon dioxide enters and leaves the leaves through the stomata, i.e., the tiny pores in the leaves. The photosynthesis consists of two parts: a light reaction and a dark reaction. During the light reaction of photosynthesis, a chain of redox reaction is performed of photosystem I (PS I) and photosystem II (PS II) that collects light energy and produces useful chemical energy products for the following CO2 assimilate reaction cycle process. This process is highly dependent on the light reaction and is independent on the amount of chemical energy provided in the plant (Kaneki, 2018, chap. 3). In the photosynthesis process, chlorophyll pigments are the most important and effective absorbers of light energy. The most abundant pigment in the majority of photosynthetic organisms is chlorophyll a (Chl a) followed by chlorophyll b (Chl b), xanthophylls, and carotenoids (β-Carotene). The photosynthesis and chlorophyll parameters (as well as physiological indices such as leaf area, height, and biomass) are used as growth indicators for the phenological and physiological developmental stages of plants (Tanaka & Tanaka, 2000; Avgoustaki, 2019). For this reason, the study of the behavior and development of a selected crop can indicate the vegetation growth of plants under healthy and stressed cultivation conditions.

In a previous study by Avgoustaki (2019), it was found that basil plants exposed to a reduced photoperiod of 14 h of continuous light did not show visible changes in their growth and development (compared to 16 h, which is considered optimal for basil). However, even if plants showed continuous development under reduced photoperiod (of 14 h), the energy grid does not provide continuous low price of electricity for the farmers to accomplish a precise shift in their production. This is the reason why we decided that it is of high importance to study plants’ response under limited and intermittent lighting system. The purpose of this research is to detect the response of basil plants under continuous light with an optimal photoperiod (of 16 light hours) as opposed to plants exposed to intermittent light and a reduced photoperiod (of 14 h). We measure the photosynthetic rate, stomata conductance as a function of CO2, chlorophyll pigments, growth indices, and light levels to assess the impact of intermittent light on basil from germination to harvest. Finally, the research aims to propose a lighting system that takes advantage of the fluctuating electricity market and shifts demand response to reduce the total energy costs without affecting the growth rate of the plants.

2. Material and methods

2.1. Plant material and growing conditions

The experiments were conducted in a indoor small-scale chamber located at the chemistry laboratory of the Department of Business Development and Technology, Aarhus University in Herning, Denmark. The dimensions of the chamber were the following: Height = 1000 mm, width = 915 mm, and length = 457 mm. The air temperature, relative humidity, and CO2 concentration were automatically monitored, using a climate control sensor (TROTEC BZ30, UK). The light in the systems came from the Budmaster II GOD-2 LED with a 90-W energy consumption. In the LED specifications, the peak wavelengths are listed as 400–480 nm (blue) and 610–720 nm (red and NIR). These remained stable throughout the experimental period.

‘Genovese’ basil (Ocimum basilicum) was selected for this study, as it is one of the plant species most commonly cultivated in vertical farms due to its high nutritional value and cultivation density, which play a key role in improving yield in vertical farming. Furthermore, sweet basil belongs to the category of long-day plants, as it needs more than 12 h of light and less than 12 h of dark in order to grow. According to previous literature, the optimal photoperiod for basil is 16 h of light (Beamen et al., 2009).

Basil seedlings filled with perlite (ISOCON Perlflor Hydro 1) were planted. Two rows and five columns of plants were used, each pot with five plants. The mean volumetric water content of the perlite at field capacity was 53–55%. The measurements started three weeks after sowing, when the plants had an average height of 8.2 cm and two pairs of actual leaves. The plants were grown in an ebb-and-flow hydroponic installation, and every second day, water enriched with the nutrients was added directly at their root zone.

To study the effects of intermittent light on crop quality and quantity characteristics, two experiments were performed since we only acquire one chamber that we could set with the desired conditions. Two consecutive experiments have been carried out from August to September and from November to December 2019. More specifically, throughout the whole first experiment the lighting conditions remained stable at 16 h of continuous photoperiod. This first experiment named GBD16L and referred as the control treatment that used as reference for comparison with the stress treatment Subsequently, we repeated the same process, where we planted the same basil cultivar (Genovese basil) in the same numbers and locations. Primarily, plants sowed and grew and continuous light of 16 h photoperiod for three weeks without being able to take measurements from leaves because of the insufficient size. Continuously, when plants entered the third week and while having one pair of leaves we started measurements under continuous light of 16 h photoperiod. When plants developed 2 pairs of fully developed leaves we altered the lighting system from 16 continuous photoperiod to 14 h of intermittent photoperiod, until the end of the experiment and the final harvest. The second experiment is considered as the stress treatment and is referred as 11D014L. During the 11D014L treatment, plants received light every 10 min per hour over a 4-h period (Table 1, Fig. 1). This system had a total of 14 h of light and 10 h of darkness per day (Withrow R.B. and Withrow A. P., 1944). The average amount of light obtained at the level of plants when the LED was turned on was expressed in Photosynthetic Photon Flux Density (PPFD), maintained at 500 µmol/m2/s, and was measured daily with the spectrometer uSpectrum (UPRtek/Licor) because of the constant increase of leaves.

The total water consumption during the 24 days of both experiments was 12 L. At the beginning of the stress treatment of 11D014L with the intermittent lighting application, the water tank was filled with 2 L of water, i.e., the maximum capacity of the tank. A total of
climate conditions (light treatments) for stress and control, respectively, in the chamber at the same time. For this reason, we first conducted the experiment under indoor conditions, and subsequently, we repeated the same experiment with the sequence described above (Table 1), creating virtually identical environmental conditions. Table 2 shows the climate conditions for each light treatment of the experiment.

### 2.2. Data collection

Prior to the experiment, the air temperature ($T_{\text{air}}$ in °C), relative humidity (RH %), and CO₂ concentration of the chamber were measured and calibrated using a climate control sensor, placed 50 cm above the crop area in the middle of the chamber to automatically log data every 10 min (TROTEC B225 CO₂ Air Quality Monitor, Germany). The $T_{\text{air}}$ and RH % values were used to calculate the air vapor pressure deficit values. Leaf temperature ($T_{\text{leaf}}$ in °C) was measured using a thermocouple attached to the leaf surface area (Solf Franc, Spain) [sensor error ± 0.03 + (0.005 × t°C)]. The measurements were performed in 30-s intervals, and the data logger recorded the average values at 10-min intervals.

The measurements of photosynthesis and chlorophyll were made manually every day in the morning at 9:00, 10:00, 11:00, 12:00, and 13:00, and in the evening at 17:00, 18:00, 19:00, 20:00, and 21:00. The sampling involved young and fully developed leaves from each plant. The portable sensor provided chlorophyll data based on the absorbance of plants at 660 and 940 nm. As seen in Table 1, the measurement from 9:00 to 17:00 coincided under the 4 h of continuous light, while the rest of the measurement took place during the 10-min light cycles.

The plants were measured every day with both manual and the automated sensors. As mentioned, the data from the gas exchange sensor and the chlorophyll content were taken manually. All the other sensors ($T_{\text{leaf}}$, Wet sensor and environmental conditions) were connected with data loggers and were taken automatically. To measure the temperature of the substrate we use WET-2 Sensor from Delta-T devices (UK) with the error of the sensor to be ±10 mS·m⁻¹. The data were logged every 10 min and the manual data tens times a day according to the schedule described above. These measurements and data acquisitions were followed by the photosynthesis and chlorophyll measurements. The sensor used to collect photosynthesis data was the LCpro-SD gas exchange sensor for portable measurement of photosynthesis (ADC BioScientific Ltd., UK) (sensor error 0.1 °C). It was calibrated before the experiment. The chlorophyll measurements were extracted using a chlorophyll sensor (CM-500, Chlorophyll Meter, Solf Franc, Spain), which was also calibrated before the experiment (sensor error ± 0.3 SPAD unit). The selected samples were young, fully developed leaves of Genovese basil.

### 2.3. Plant biomass measurements and evaluation of the electrical energy used for basil production

The fresh mass of the shoots (the stems, flowers, and leaves) was measured. For the dry mass measurements, the shoots were placed in an oven at 80 °C for 24 h during which all the water evaporated.

The input of the electrical energy was measured using the Clairvin Arnoux PEL 103 power and energy logger. The electrical energy consumed for the basil production was measured for each treatment, taking into account the total leaf biomass produced by the eight plants,

### Table 2

The climate conditions used for growing basil plants in the small-scale chamber.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (days)</th>
<th>Relative Humidity (RH %)</th>
<th>VPD (Pa)</th>
<th>$T_{\text{air}}$ (°C)</th>
<th>$CO_2$ (ppm)</th>
<th>Energy Demand (kWh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O8D16L</td>
<td>23</td>
<td>29.5 ± 5.6</td>
<td>2190 ± 315</td>
<td>24.6 ± 1.3</td>
<td>458 ± 10</td>
<td>71.57 ± 5.18</td>
</tr>
<tr>
<td>110D14L</td>
<td>23</td>
<td>36 ± 2.3</td>
<td>1586 ± 88</td>
<td>21 ± 0.9</td>
<td>443 ± 13</td>
<td>57.63 ± 2.26</td>
</tr>
</tbody>
</table>
the length of each treatment, the photoperiod (hours of light), and the energy consumed by the system per hour. The result is presented in kWh kg\(^{-1}\).

2.4. Statistical analysis

Data were subjected to an independent-samples t-test, including if they followed Levene’s test for homogeneity of variances. The analyses were performed using SPSS for Windows (IBM Statistics for Macintosh, version 25.0).

2.5. Hypothesis

If we reduce the photoperiod that basil plants receive in an indoor environment, and we apply an intermittent light treatment after the germination of the plants, the growth and development of the photosynthesis rate will not be reduced.

3. Results

For the statistical analysis of our dependent variables, we performed independent sample t-test under the two different independent variables of the lighting treatments (CBD161L and I10D14L). However, for sample size we used only the final 14 days in both experiments that had diversified lighting conditions. The previous 10 days of measurements, since the conditions were the same in both experiments were statistically analyzed and presented no significant difference. This is the reason why they were not included in the data analysis but they presented concisely in Table 3.

The data were initially tested for the homogeneity of the variance under Levene’s test. The Levene’s test can reject or accept the hypothesis of equal variances of the data, where F expresses the distribution of data at N-k degrees of freedom (df) at a significance level of α = 0.05. In the text is described by the following format F (df) = F- statistic, p = p-significance value. Subsequently, an independent samples t-test was performed for each dependent variable to determine whether there is a statistical significant difference between the mean values of the lighting treatment groups. The result of the independent samples to compare the means for the two lighting treatments (CBD161L – I10D14L) for all the dependent variables separately. In the report we included the t-statistic value (t-test), the degrees of freedom (df) and the value of significance of the test (p-value), using the format: t (df) = t-test, p = p-value.

3.1. Development and study of abiotic indicators under different light treatments

An independent-samples t-test was performed to compare the two light treatments (CBD161L and I10D14L) in order to determine whether there is a statistically significant difference between the means of various growth indicators for the basil plants grown in the small-scale indoor chamber (Tleaf, Tsub, CGI, °C) chlorophyll pigments, and As) (Fig. 3).

We examined multiple growth indicators of the basil plants, e.g., Tleaf in the control treatment (N = 14) was compared with Tleaf was (23.2 ± 0.9) °C, and the stress treatment (N = 14) was compared with Tleaf was (18.75 ± 1) °C. An independent-samples t-test was conducted to test if the two treatments showed statistically significant different means in terms of Tleaf. The assumption of homogeneity of variances was tested and satisfied using Levene’s F test, F (26) = 1.371, p = 0.252. The independent-samples t-test was associated with a significant effect t (26) = 12.682, p < 0.001. Thus, the CBD161L treatment was associated with a statistically significant different mean than the I10D14L treatment. Cohen’s d was estimated at 0.5, which is a medium effect based on Cohen’s (1992) guidelines. The differences in the values of Tleaf and Tsub that can observed in the first 11 days of the two experiments in control conditions have no significance value.

There was a significant difference in the temperature of the hydroponic substrate (Tsub) in the CBD161L treatment Tsub was (21.5 ± 0.6) °C and the I10D14L treatment Tsub was (19.7 ± 0.5) °C with N = 14 for both cases. Thus, the homogeneity of variance was violated, and we proceeded without an assumption of equal variances: F (26) = 0.165, p = 0.688. An independent-samples t-test showed no statistically significant difference with t (26) = 8.359, p < 0.001. These results suggest that the light treatment had a major effect on the Tsub for basil plants.

3.2. Statistical analysis of photosynthesis

During photosynthesis, green plants capture light energy, carbon dioxide, and water and convert these into oxygen and energy-rich organic compounds. In this research, a portable gas exchange sensor (LCpro-SD) was used to estimate the photosynthesis process and its evolution during the experiment with the basil crop. Using the sensor, we monitored the photosynthesis rate (As = μmol m\(^{-2}\)s\(^{-1}\)) (Fig. 4).

The CBD161L treatment As = (9.44 ± 1.2) μmol/m²/s was compared with the I10D14L treatment As = (6.79 ± 0.7) μmol/m²/s with N = 14 in both samples to test the difference in the As between the healthy and stressed plants. To examine whether As had a higher level of statistical significance with intermittent light, we performed an independent-samples t-test. Levene’s test for equality of variance was run to verify the assumption of homogeneity of variances, and equal variances were not assumed: F (26) = 4.181, p = 0.05. The independent-samples t-test was performed with a statistically significant effect, t (20.694) = 6.936, p < 0.001. Cohen’s d was estimated at 1.5, which is a large effect based on literature.

As mentioned previously, we performed photosynthesis measurements every day: five times in the morning hours and five times in the evening hours. The measurements made during the intermittent treat-
ment took place during the 10-min light cycles, but also at the end of the 4 h of continuous light (Table 1). Accordingly, we tested both the evolution of As during the 10-min light cycles with limited light radiation as well as after the 4 h of continuous light, which allows plants to receive, absorb, and process more light energy and convert the energy into chemical energy.

We therefore decided to perform a statistical analysis of the 10-min periods and the 4-h periods between the CBD16L treatment and in the I10D14L treatment.

The CBD16L treatment $A_{510_{min}} = (9.44 + 1.2) \mu$mol/m²s for the 10-min period measurements of photosynthesis ($A_{510_{min}}$) was compared with the corresponding measurements of the I10D14L treatment $A_{510_{min}} = (6.29 + 0.8) \mu$mol/m²s with $N = 14$ in both samples, which found that there was statistical significant difference between the two groups: $t(26) = 7.830$, $p < 0.001$ by performing an independent samples t-test. Since the assumption of homogeneity of variances was not violated using Levene’s test, we continued with the assumption of equal variances at $F(26) = 1.655$, $p < 0.210$. These results suggest that intermittent light has a significant effect on the photosynthesis of plants at measurement levels of 10 min. Cohen’s $d$ was also calculated at 2.9 showing a strong effect.

Next, we compared the difference between the photosynthesis during the 4-h period of light ($A_{4_{hour}}$) in both the continuous treatment - CBD16L with $A_{4_{hour}} = (9.44 + 1.2) \mu$mol/m²s and the intermittent treatment - I10D14L with $A_{4_{hour}} = (10.28 + 1.1) \mu$mol/m²s with $N = 14$ in both samples. Based on the Levene’s test of homogeneity of variance, the results are $F(26) = 0.266$, $p = 0.611$, showing that our variances are equal. An independent-samples t-test was performed under the two treatments. There was no significant effect of intermittent light on $A_{4_{hour}}$ at the level $p < 0.05$ for the two treatments at $t(26) = -1.864$, $p = 0.074$. The negative mean differences indicate higher mean of the I10D14L treatment than the CBD16L treatment. Our results show that plants under intermittent light continued to develop steadily as the plants under continuous light. More specifically, 4 h of intermittent light was sufficient to develop the plants’ photosynthetic capabilities and absorb enough light energy to grow. Conversely, when the plants only received 10 min of light, they did not receive the energy needed to perform photosynthesis. Even when $A_s$ was reduced because of the intermittent light, the plants in the I10D14L treatment showed no significant difference in the photosynthetic rate during the 4 h interval in comparison with the photosynthetic rate of the CBD16L treatment (Fig. 2b).

![Fig. 2. Daily electricity consumption during a) continuous light treatment (CBD16L) and b) intermittent light treatment (I10D14L).](image)

![Fig. 3. Average daily evolution of [a] leaf temperature ($T_{leaf}$ in °C) and [b] substrate temperature ($T_{sub}$ in °C) for healthy plants (blue line) and stressed plants (orange line). Days 1–11: a) CBD16L (control/continuous light); b) I10D14L (control/continuous light); days 12–24: a) CBD16L (control/continuous light); b) I10D14L (stress/intermittent light). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)](image)
The analysis of the stomata conductance (gs) between the C8D16L treatment is $g_s = (0.071 + - 0)$ mmol m$^{-2}$ s$^{-1}$ and the I10D14L treatment with $g_s = (0.077 + - 0)$ with $N = 14$ in both samples, showed a no statistically significant difference at $t (26) = -1.394$, $p = 0.175$ (two-tailed). As can be observed from the climatic conditions in the growth chamber, there is a negligible increase in the CO$_2$ and vapor pressure deficits (VPD) of our experiment that could have caused the increase in stomatal conductance between the two treatments. Furthermore, the increase in stomatal conductance following the 10-min cycles is also worth mentioning.

3.3. Statistical analysis of chlorophyll

For data collection, the portable LCpro-SD sensor was used to measure the chlorophyll content index (CCI) of the basil plants. As mentioned above, the data acquisition during the experiment was conducted in specific time periods as presented in Table 1.

In Fig. 5, the evolution curve of CCI under the two light treatments is presented. As can be observed, the curve shows an aligned development of chlorophyll production for the plants subjected to the stress treatment with 14 h of photoperiod and intermittent light (I10D14L) and the plants following the control treatment (C8D16L). Measurements obtained from the C8D16L treatment is CCI = (33.16 + - 4.6) and the I10D14L treatment is CCI = (36.33 + - 3.2) with $N = 13$ in both samples, showed no statistically significant difference between the two treatments, $t (26) = -2.016$, $p = 0.055$. As the experiment progressed, the chlorophyll values continued to increase until the end of the experiment, reaching 39.82 μmol/m$^2$. More specifically, the chlorophyll concentration of the healthy plants (C8D16L) varied between 27.7 and 39.82 during the stress days of the experiment (days 12–24). (The last day- No25 of the second experiment the CM-500 Chlorophyll Sensor presented malfunctions, so we could not take the last day's data of I10D14L to compare them with C10D14L).

Using the research findings of J.F.C. Gonçalves et al. (2008), we calculated the concentration of Chl a, Chl b, Chl tot, and a (the fraction
of PAR absorbed by the leaf of the plants) with the following equation:

\[
\begin{align*}
\text{Chl} a &= -421.35 + 375.02 \times (\text{CCI})^{0.1863}, \\
\text{Chl} b &= 38.23 + 4.03 \times (\text{CCI})^{0.88}, \\
\text{Chl}_{\text{tot}} &= -283.2 + 269.96 \times (\text{CCI})^{0.277}, \\
\text{a} &= -3.50 + 3.96 \times (\text{CCI})^{0.027},
\end{align*}
\]

where \(\text{CCI}\) is the measurement of the portable chlorophyll meter (CM-500).

We compared the daily mean values of the two treatments. An independent-samples \(t\)-test was performed to compare the effects of the intermittent light on the plants’ chlorophyll pigments in the two treatments.

\(\text{Chl} a\) is one of the most important chlorophyll pigments (in combination with \(\text{Chl} b\), as they differ minimally in their structure) [Stearns & Olson, 1958]. A crucial property of \(\text{Chl} a\) is the versatility, enabling active participation in several functions of the photosynthetic process, including photon capturing, transfer and storage of photons, and energy storage at the antennas (Oxboorouh, 2000). \(\text{Chl} a\) absorbs radiation in the red and blue nanometers of the light spectrum (Fedor, 2008). Apart from \(\text{Chl} a\), plants use other pigments (\(\text{Chl} b, c\), carotenoids, and phycobilins), which absorb radiation with intermediate wavelengths. This process makes better use of solar energy (Avgoustaki, 2019) (Fig. 6).

In order to examine the differences in the \(\text{Chl} a\) between the plants in the control and stress groups, an independent-samples \(t\)-test was conducted for each one of the dependent variables. To start with \(\text{Chl} a\), Levene’s test for homogeneity of variances was satisfied with \(F(24) = 2.300, p = 0.142\). The results of the \(t\)-test indicated that there was no significant difference in \(\text{Chl} a\) between the two treatments, \(t(24) = -1.956, p = 0.062\). These results suggest that the plants in the \(\text{C8D16L}\) treatment with \(\text{Chl} a = (297.7 \pm 20)\) had a similar \(\text{Chl} a\) content compared to the plants in the \(\text{I10D14L}\) treatment with \(\text{Chl} a = (310.7 \pm 13)\) with \(N = 13\) in both samples.

Subsequently, the \(\text{Chl} b\) for the two treatments was analyzed and compared. The independent-samples \(t\)-test indicated that the \(\text{C8D16L}\) treatment with \(\text{Chl} b = (125.9 \pm 11)\) showed smaller mean values in comparison with the \(\text{I10D14L}\) treatment with \(\text{Chl} b = (133.3 \pm 7.5)\) with \(N = 13\) in both samples, \(t(24) = -2.007, p = 0.056\) (two-tailed) with no significant statistical difference.

The \(\text{Chl}_{\text{tot}}\) measurements of the \(\text{C8D16L}\) treatment with \(\text{Chl}_{\text{tot}} = (427.4 \pm 29)\) and the \(\text{I10D14L}\) treatment with \(\text{Chl}_{\text{tot}} = (446.5 \pm 17)\) with \(N = 13\) in both samples) did not show a statistically significant effect of the intermittent light at \(t(24) = -1.963, p = 0.061\).

An independent-samples \(t\)-test was performed to find \(a\) (the fraction of PAR absorbed by the leaf) of the healthy plants and the stressed plants exposed to intermittent light. The results from the independent-samples \(t\)-test indicated that the \(\text{C8D16L}\) treatment with \(a = (0.85 \pm 0)\) showed similar mean values compared to the \(\text{I10D14L}\) treatment with \(a = (0.86 \pm 0)\) with \(N = 14\) in both samples, with no significant different results \(t(24) = -1.944, p = 0.064\) (two-tailed).

3.4. Quality and physiological evaluation of basil grown under different lighting conditions

The length of the continuous light was ten days in both treatments. After a restitution day, we modified the light treatment in the \(\text{I10D14L}\) group with intermittent light for the next 13 days. The \(\text{C8D16L}\) group continued to grow under continuous light, allowing us to examine the difference in the performance of the two treatments. The control treatment with the continuous 16 h of light was shorter when we started the first measurements after the germination period had elapsed (i.e., after the third week), and the plants had a sufficient leaf size area for measurement (Table 4a). After ten days, the plants had received enough light for their optimal growth, and we were able to start the stress
Table 4a Results of statistical analysis of the mean values of quantity assessment first 10 days in C8D16L and I10D14L were both followed continuous lighting of 16 h. The columns present Sample size (N), Mean values (Mean), Standard Deviation (SD), t (t-test), Degrees of Freedom (df), level of significance (p-value).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C8D16L</td>
<td>8</td>
<td>19.3</td>
<td>1.7</td>
<td>-0.138</td>
<td>14</td>
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<tr>
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<td>19.5</td>
<td>1.8</td>
<td>-0.146</td>
<td>14</td>
<td>0.886</td>
</tr>
<tr>
<td>LA (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8D16L</td>
<td>8</td>
<td>85.6</td>
<td>27</td>
<td>-0.431</td>
<td>14</td>
<td>0.673</td>
</tr>
<tr>
<td>I10D14L</td>
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<td>87.5</td>
<td>24</td>
<td>-0.383</td>
<td>14</td>
<td>0.708</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8D16L</td>
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<td>12.3</td>
<td>2.6</td>
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<td>14</td>
<td>0.673</td>
</tr>
<tr>
<td>I10D14L</td>
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<td>12.8</td>
<td>1.9</td>
<td>-0.383</td>
<td>14</td>
<td>0.708</td>
</tr>
<tr>
<td>Fresh Mass (g)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8D16L</td>
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<td>1</td>
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<td>0.675</td>
</tr>
<tr>
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<td>1.2</td>
<td>-0.383</td>
<td>14</td>
<td>0.708</td>
</tr>
<tr>
<td>Dry Mass (g)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8D16L</td>
<td>8</td>
<td>0.34</td>
<td>0.2</td>
<td>-0.199</td>
<td>14</td>
<td>0.845</td>
</tr>
<tr>
<td>I10D14L</td>
<td>8</td>
<td>0.36</td>
<td>0.2</td>
<td>-0.199</td>
<td>14</td>
<td>0.845</td>
</tr>
<tr>
<td>Fresh Biomass (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.38</td>
<td>0.4</td>
<td>-0.428</td>
<td>14</td>
<td>0.673</td>
</tr>
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<td>I10D14L</td>
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<tr>
<td>Dry Biomass (g)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C8D16L</td>
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<td>0.845</td>
</tr>
<tr>
<td>I10D14L</td>
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<td>0.15</td>
<td>0.07</td>
<td>-0.199</td>
<td>14</td>
<td>0.845</td>
</tr>
</tbody>
</table>

The total biomass of the plants was estimated by the equations:

Fresh biomass = fresh mass (g) / cultivation area (m²) (5)

Dry biomass = dry mass (g) / cultivation area (m²) (6)

Table 4b Results of statistical analysis of the mean values of quantity assessment of the 14 stress days between C8D16L and I10D14L. The columns present Sample size (N), Mean values (Mean), Standard Deviation (SD), t (t-test), Degrees of Freedom (df), level of significance (p-value).

Fig. 7 shows the electrical energy necessary for the total crop production in each treatment. The conditions applied in the I10D14L treatment produced the highest biomass production with a reduced electrical energy input compared to the C8D16L treatment. As presented in Table 2, the independent-samples t-test indicated that there was no statistical significant difference in the mean dry biomass (g m⁻²) for the two treatments. Finally, we could observe a statistically significant difference in the energy demand of both treatments, caused by the reduced photoperiod.

4. Discussion

One of the aims of this research was to investigate the sustainability and applicability of indoor cultivation systems to create a more affordable and ecological production for plants, mainly to address the urban demand for fresh food. In an indoor urban farm, the majority of the energy is consumed for lighting the premises, making it the most costly process of running an urban farm. Therefore, by reducing the amount of light/photoperiod (Avgoustaki, 2019) without reducing the growth rate of the plants, the purpose was to decrease the operational costs of indoor urban farming.

The main purpose of this study was to optimize the light conditions for growing basil indoors by alternating the light from a continuous to an intermittent flow and identifying the plants’ response to the light treatments. More specifically, our goal was to examine the difference in photosynthesis between the two treatments as well as and for how long we could shift the demand response to more cost-effective energy without affecting the yield and the quality of the crop.

Abiotic factors like light, CO₂, water, and nutrients as well as physiological factors such as stomatal conductance and transpiration can strongly influence the photosynthetic activity of plants and their optimal growth. In this study, we followed the growth, pigment concentrations, gas exchanges, and photochemical efficiency in basil plants to detect their response to intermittent light.

4.1. The effect of different light treatments on basil quality, leaf functions, and physiological parameters

Throughout all the phases of the experiment, the leaf temperature (Tleaf) was sufficiently lower than the air temperature in the chamber. According to Gimnez and Thompson (2005), this difference indicates a healthy water status of the canopy sourcing from the cooling effect caused by the evaporation of the plants. Furthermore, Tleaf is closely related to incidental radiation and vice versa (Jones, 1985). Based on Fig. 1 [a], the difference between the two treatments can be explained by the reduced thermal radiation reaching the leaf surface under the intermittent light.

In addition, the substrate temperature (Tsubstr), or the root zone temperature, plays an important role in the water and nutrients uptake of the plants. This is because Tsubstr can alter the responses of the plant shoots by affecting the temperature of a shoot apical meristem (McMaster and Wilhelm, 2003) and continuously regulate the hormonal balance in water and nutrient uptake (Bhatiacharya, 2019).

Stomatal conductance (gs) allows the stomata to absorb CO₂ and can be used for evaluating the plant water status. In other words, the role of stomata is to control the leaf transpiration and maintain the leaf water status by opening and closing the stomata (Moriana et al., 2002). According to Kirschbaum and Pearly (1987), stomatal conductance is an important factor in the photosynthetic induction response of a leaf. In systems with no limitation and controlled CO₂, the photosynthetic induction has a duration of a few minutes, whereas plants with unstable, lower concentrations of CO₂ require at least half an hour to complete the induction process, which is closely correlated with stomatal conductance. Stomatal conductance is highly affected by VPD and tends to decrease when there is an increase in CO₂ in the cultivation area (Field C. B., 1995). However, the graph of stomatal conductance (Fig. 2 [d]) shows that the transpiration rate of the leaves...
was stable and with a significant increase in the water level and absorbed CO₂ of the basil leaves (Gimnez & Thompson, 2005). Furthermore, the significant increase under the intermittent light indicates that the plants can successfully absorb CO₂ and traverse it via the epidermal cell layer, at the photosynthetically active leaf mesophyll cells.

Light is probably the most crucial factor that can affect the growth and development of green species as well as the production and the biomass levels. A plant’s response to light can provoke physiological alterations that can affect the CO₂ assimilation and optimization of gas exchanges in the plant. Furthermore, light plays an important role in the quantitative and qualitative process (Gonçalves et al., 2008). For this reason, it is important to examine the enhancement of photosynthesis under continuous light compared to photosynthesis under intermittent (fluctuating) light. Grobbelaar et al. (1996) stated that longer and continuous dark periods do not necessarily lead to higher photosynthetic rates and efficiency. According to Iluz, Alexandrovich, and Dubinsky (2012), plants absorb all the necessary light during the light period (gross photosynthesis) and use it continuously in the dark period. More specifically, an increase in light intensity can reduce growth and photo-inhibition (significant loss of photosynthetic production) due to the production of damaging reactive oxygen intermediates. Fig. 2 [b] and [c] show the differences in photosynthetic rate measurements of the plants under intermittent light. As can be observed from the graphs, there was a significant difference in the measurement of As for the 10-min light period compared with the measurement of the 4-h light period, which did not show significant differences under continuous light. This can be explained by the fact that cells are not able to generate enough energy through the photosynthesis process in just 10 min to meet their metabolic requirements. However, during the 4-h period, the cells managed to process enough energy via photosynthesis, which shows that plants can increase As without affecting the primary production. Further research is needed to uncover the dark periods.

Photosynthesis is the process by which plants modulate the function and the structure of the photosynthetic device at growth irradiance. According to Adams et al. (1999), during short-term photoacclimation (seconds to minutes), a heat dissipation of the excess excitation energy through carotenoids was observed, which can influence the distribution of absorbed light energy between PSI and PSII. Further research is needed to analyze the carotenoid level under intermittent light in detail. Another important point that can explain our results is the Rubisco model, which is a useful tool for clarifying the intermittent light that can increase the photosynthetic efficiency. More specifically, from the experiment, we can deduce that the number of artificial photosyntheses generated in the photon reception process determine the discrete Rubisco particles circulating in the Calvin cycle as well as their speeds in the cycle.

The measurements of various chlorophyll pigments from chloroplasts and their analyses were used as stress indicators of high irradiance in the plants (Gonçalves et al., 2005). Based on previous literature (Tzina et al., 1987), the amount of the chlorophyll accumulation in the early growth stages of the plants depends on the total radiation received by the crop, independent of the intervals during the dark period of the photoperiodic cycle. Furthermore, as previously mentioned in this study, the rate of photosynthesis for intermittent light depends on the inter-polation between the dark intervals. When exposed to shorter dark intervals, plants contain larger photosystems units, i.e., large protein complexes embedded in the thylakoid membrane for absorbing and converting solar energy. Fig. 3 depicts that the chlorophyll pigments followed an increasing rate throughout the experiment, indicating that the plants had a stable and increasing rate if they absorbed the given radiation. In the short dark periods of intermittent light, thylakoids with few and large-in-size photosystems were found, while the thylakoids found in the longer dark periods were more and smaller in size to maintain the same accumulation rate of chlorophyll.

4.2. Effect of different lighting system on biomass accretion and energy demand

Photoperiod and light distribution are vital in plant biomass production, leaf size, and leaf area (Adams & Langton, 2005). Initially, the treatment with the continuous light and the longer photoperiod was expected to result in higher biomass production of basil plants. However, in Fig. 4, we can observe an increase in the shoot dry biomass with the I10D14L treatment, without any statistical difference compared to the C8D16L treatment. At the same time, the energy demand in kWh was significantly lower [t (23.624) = 25.961, p = < 0.001] under the intermittent treatment. The dry shoot biomass in the I10D14L treatment averagely consumed less energy (12.48 kWh) than the C8D16L treatment. From an economic perspective, the electricity price in Denmark (where the experiment took place) is around 0.3123 €/kWh, giving us an energy cost saving of 1.84 €/m²/day with a 90-W...
LED. Further research on larger vertical farms with more LED installations and greater energy loss is needed.

Moreover, it was found that a shorter photoperiod and intermittent light slightly reduced the fresh mass and the leaf area of the plants but without any statistical significance. This may be due to the lower radiation that the basil plants received during the ING14t treatment, reducing the nitrate concentration and at the same time increasing the amount of chlorophyll and carotenoids contained in the plants (Avgoustaki, 2019). Beaman et al. (2009) observed that a fresh biomass production with a higher carotenoid concentration and lower the nitrate content results in high-nutrient products.

In this research study, we focused on measuring the energy input in a small-scale cultivation area consumed for different treatments. Our goal was to identify energy savings depending on light conditions as well as to reduce the energy production costs in indoor cultivation systems. Based on the data collection from this experiment, the yield of the treatment with intermittent light was the same as that of a commercial product, but with a lower energy costs compared to the continuous light treatment.

5. Conclusions

Cultivation of basil in a small-scale growth chamber under intermittent lighting showed a positive effect on the growth, development, quality, and quantity of the plants compared with the control conditions of continuous lighting. The plants grown under intermittent light with a reduced photoperiod had a positive effect on the final biomass production. In addition, the daily measurements used to monitor the rate of photosynthesis and chlorophyll content of the plants showed no negative results. The photosynthetic rate of the plants continued to grow during the days of the experiment when the plants were exposed to shorter light intervals (4 h). Furthermore, the chlorophyll content and the chlorophyll pigments maintained a steadily increasing rate throughout the plants' entire growth process. The purpose of this experiment was to test the response of basil plants under intermittent light as well as to examine their growth rate and quality using an energy-efficient production system for indoor farming. The results of the experiment suggest an electricity system in vertical farms based on energy prices across countries. The research shows that vertical farms can design their lighting system, adjusting the consumption based on low or high electricity prices. A potential taste assessment of plants under different lighting systems would be an interesting addition to the process. Future research is needed to investigate the response of plants under various durations of intermittent light and with various LED spectrum combinations.

Vertical farms are a great opportunity to design more sustainable systems using innovative technologies to meet the urban demand. However, since these systems still present new opportunities for additional energy optimization, it is crucial to develop smarter, more efficient applications and techniques, enabling vertical farms to produce high-quality products while minimizing costs.

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Uncited references

Barbosa et al., 2015; Schmidt et al., 2010.

CRediT authorship contribution statement

Dafni Despoina Avgoustaki: Data curation, Formal analysis, Writing - original draft. Jinyue Li: Data curation. George Xydias: Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

