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Development of a dynamic protocol for improving the productivity of soilless farming systems

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ABSTRACT

Climate change and population increase are becoming a threat to human feeding. New technologies and practices are under development, and a significant effort is being put into developing indoor farming, which allows for all-year-round production of high-quality food, regardless of the climate. Moreover, indoor farming promises extreme water and chemical usage reduction, specifically when the system is autonomously regulated with an IoT architecture. Despite these attractive characteristics, indoor systems require considerable energy to provide adequate temperature and lighting for cultivated crops. This demand is often high enough to make the production system economically unsustainable. This work aims to develop a cultivation protocol for baby lettuce plants (up to three weeks old plants) that can increase overall productivity while mitigating the issue of high energy demand. To this aim, we performed a Design of Experiment to assess crop responses to different levels of nutrients, temperature, and light intensity with the productivity of the system and the quality of the harvested product. The collected data were used to design a dynamic cultivation protocol, which defines different growing conditions according to the plant development stage. Results demonstrate that the dynamic protocol can enhance system productivity by up to 25 % in biomass accumulation, compared with the productivity obtained with fixed growing conditions, while maintaining the same high quality. Furthermore, the improvement is achieved without increasing the resource use, confirming the potential of this approach to enhance the economic sustainability of indoor soilless farming.

1. Introduction

Soilless farming is a method of growing plants without soil. The concept dates to ancient civilizations, as the Babylonians and Aztecs showcased the potential of growing plants using nutrient-rich water [29]. Today, soilless farming has evolved into modern and efficient indoor farming techniques, such as hydroponics, aeroponics, and aquaponics, which have a high production potential, especially in geographical areas where the climate is particularly severe, or some resources are rare (e.g. water or space) [2,81]. Indeed, in recent years, several companies entered the agrifood market with soilless-produced vegetables using high-tech soilless production systems (SPSs) [17]. Often, SPSs grow crops indoors in vertically stacked layers as an alternative to traditional horizontal farming [75]; this practice goes under the name of vertical farming (VF) or VF production systems (VFPSs), which can maximize space efficiency [40,75]. SPSs aim to produce vegetables in urban settings or wherever conditions are unfavorable for traditional agriculture while saving water, pesticides, and other chemicals. Also, SPSs have a higher yield per unit area, reduced water and land use, and the ability to grow crops year-round in a protected environment [5,64]. Moreover, they have the potential to contribute to sustainable urban food production by reducing the environmental impact associated with long-distance transportation [45,64]. On the other hand, SPSs have a considerably higher energy demand because they need artificial lighting and temperature control [45]. Despite their high energy requirements [45], SPSs have the potential to become an important source of vegetable production. They require efficient IoT-based monitoring and control of the production parameters to ensure their sustainability and economic viability [43,60,28].

Resource use efficiency is crucial in vertical farm economic and environmental sustainability [69]. In this context, integrating IoT-based monitoring and control systems is widely recognized as a critical enabler for optimizing resource use and production processes in SPSs [67]. The development of SPSs technology is rapidly evolving, and more research

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on automated systems is needed to achieve a higher level of optimized production. In recent years, the technology related to SPSs rapidly evolved from a basic sensor/actuator system [14,21] to a digitalized entity able to mime and predict system outcomes [10].

Lettuce (*Lactuca sativa*) is an economically significant crop, being one of the most commercially valuable leafy vegetables [23]. It is highly versatile and can be grown in a variety of production systems, including field crops, greenhouses, and indoor SPSs. Its fast-growing nature makes it a profitable crop for producers [8] as well as an interesting crop for academic research [23,27].

So far, the majority of published studies have focused on optimizing one or a few specific parameters, such as nutrient solution [35,53], lighting [56,57], temperature [64], and so on. However, a comprehensive approach investigating the interplay between multiple environmental factors is still lacking. Moreover, the effect of such interplay between factors should be investigated on diverse SPSs outputs [33], using an adequate set of key performance indicators [32]. Indeed, the analysis of a single output may limit the overall understanding of the performance of the entire system, whereas a methodology able to analyze multiple outputs will provide a robust understanding of an SPS.

Even though we did not find any research explicitly applied to maximize SPSs productivity, we identified some research papers that paved the ground for this application. For instance, [30] and [55] revealed the impact of dynamic environmental control on plant growth within controlled agricultural systems. Temperature variations, especially between day and night, play a pivotal role, with higher daytime temperatures associated with increased plant height in different crops.

In addition to temperature, diurnal shifts in light intensity influence plant acclimation at physiological and morphological levels [49,78]. Research indicates that stable and fluctuating light intensities during photoperiods produce comparable effects on growth and photosynthesis in some species, while intermittent night illumination further demonstrates plants' adaptive response to light variations [13,39,41,82]. Another aspect of lighting is light interception, which is critical in controlled environments; gradually increasing daily light integral throughout the growth period encourages early-stage leaf expansion, enhancing canopy-level photosynthesis [12,38].

Considering nutritional parameters, dynamic electrical conductivity (EC) management in nutrient solutions has similarly shown to enhance growth, with low daytime EC and high nighttime EC in tomato cultivation, increasing yield and biomass while reducing blossom-end rot [77].

Humidity levels also influence growth through effects on transpiration and organ elongation, with temporary increases in humidity enhancing elongation rates. However, prolonged exposure heightens disease risk and reduces stomatal response, increasing post-harvest wilting susceptibility [31,61,68].

Even though the reported researches focus on daily-based dynamic parameters, they highlight that dynamic growing conditions can optimize plant development, advancing both efficiency and sustainability in indoor SPSs [38]. Indeed, [38] proposed a dynamic approach to the management of SPSs, aiming to optimize resource use efficiency, product quality, and energy costs by continuously adjusting environmental factors in response to both changes in plant physiology and external factors such as electricity prices.

Our research integrates into this context, focusing on the productivity of the SPSs, particularly exploring the dynamic conditions of crop development stages (e.g. early/mature stages) rather than on a day/night basis, seeking which growing condition better meets crop needs week by week. Here, we define a growing condition as a set of environmental factors (e.g. temperature, humidity, lighting).

Based on these considerations, the following research questions were formulated:

RQ1: Does the crop respond differently to the same environmental factors at different stages of its growth?

RQ2: If so, can a dynamic protocol increase crop production compared to fixed growing conditions?

These questions served as bases for the study presented in this article.

This paper is structured as follows: Section 2 describes the material and methods used to obtain the data needed to construct the dynamic protocol. Whereas Section 3 reports dynamic protocol results. Section 4 discusses our findings and answers to the research questions, while Section 5 concludes the paper.

2. Material and methods

To investigate how crops respond to different growing conditions, an aeroponic chamber for lettuce cultivation was developed and used to collect data. Different combinations of fixed environmental factors were exploited, performing a full Design of Experiment (DoE [9]). The collected data were used to define a dynamic protocol, where the environmental factors are changed on a weekly basis. The dynamic protocol was then tested in the aeroponic chamber to compare its results with those obtained using the fixed growing conditions. The results were finally used to answer the research questions.

2.1. Aeroponic chamber for lettuce cultivation

2.1.1. System construction and IoT development

The system construction was a meticulous process, ensuring every detail was considered to enhance the reliability of the research. Three commercial aeroponic base modules (EDO Radici Felici Srl, LT, Italy) were fully sensorized and automatized. Each system includes a reservoir tank (measuring 35 cm in height, 70 cm large, 45 cm wide, allowing a cultivable surface area of 2400 cm^2), an irrigation pump (Xylem Flojet, 10.5 bar, 6 liters/min, see Table 1), a pipe system with seven nozzles to spray the nutrient solution onto the plant's roots, and a frame able to hold both the tank and the LEDs. White light LEDs, with 17 % blue, 45 % green, and 38 % red proportions, were provided by ELMO srl (PV, IT, see Table 1), and the frame allowed for adjustable light positioning. Each system had three LED bars, and each of them was powered by 12 V, 3A. The whole structure was placed within a growth tent (Mammthtent, NL) measuring 1.5 m times 1.5 m

Each tank was equipped with EC and pH sensors (Atlas Scientific, NY, USA, see Table 1), as well as a water temperature sensor (Dallas Semiconductor, TX, USA, see Table 1). Within the tent, a CO_2 sensor (Atlas Scientific, NY, USA, see Table 1) and a temperature/humidity sensor (Adafruit Industries, NY, USA, see Table 1) were steadily placed in a convenient location, and a set of actuators was also connected. The list includes four peristatic pumps (Atlas scientific, EZO PMP, NY, USA, see Table 1) to adjust the nutrient solution (macro-nutrients, micro-nutrients, pH increase, and pH decrease), a heater (Solea, booster heater, see Table 1) and a cooler (De Longhi, Pinguino, see Table 1) to maintain the correct temperature, an exhaust fan (Blauberg, bi-turbo 15 cm, see Table 1) to decrease humidity. Within the tent, ventilation was provided using a continuously active small oscillator fan.

We defined the sensorized aeroponic system contained within the tent as the cultivation chamber.

A Raspberry Pi (RPi), a single-board computer, was used for data acquisition and system control. The previously described sensors could communicate with the RPi using two communication protocols: Dallas 1-Wire protocol [36], for the water temperature sensor and I^2C [48], for all the other sensors. The first one is known for its cost-effectiveness, low complexity, and high interoperability due to its open-standard design, while the latter uses a two-wire serial communication standard (SDA and SCL) that minimizes hardware requirements while enabling easy integration of multiple devices on a single bus.

Fig. 1 shows a schematic representation of the electronic system, while Fig. 2 shows one of the cultivation chambers.

An open-source software - MyCodo ([54].) - was installed onto the

Table 1
Summary of actuators and sensors utilized in the cultivation chamber.

Sensor	Carbon Dioxide	Nutrient Solution pH	Nutrient Solution EC	Temperature	Humidity	Water Temperature
Range	0 - 10,000 ppm	0 - 14	0.07 µS/cm – 50,000 µS/cm	−40 °C - +80 °C	10 % - 99 %	−55 °C - +125 °C
Minimum response time	1 s	800ms	1 s	2 s	8 s	750ms
Actual data collection time	15 s	60 s	60 s	60 s	60 s	300 s
Resolution	1 ppm	0.001	0.01 µS/cm	0,05 °C	0,05 %	0.02 °C
Accuracy	5 %	3 %	2 %	0.5 %	2 %	5 %
Data protocol used	I^2C	I^2C	I^2C	I^2C	I^2C	Dallas 1-wire
Operating Voltage	3.3 V - 5 V	3.3 V - 5 V	3.3 V - 5 V	3.3 V - 5 V	3.3 V - 5 V	3.3 V – 5.5 V
Positioning in the prototype	Above cultivated area	Inside cultivation box	Inside cultivation box	Above cultivated area	Above cultivated area	Inside cultivation box
Brand	Atlas scientific	Atlas scientific	Atlas scientific	Adafruit	Adafruit	Dallas semiconductors
Compliance		ISO 10,523	NSF / ANSI 51			

Actuators	**LEDs**	**Irrigation Pump**	**Peristaltic pumps**	**Cooler**	**Dehumidifier**	**Heater**
Nominal power	75 W	72W	2W	1500W	23	250W
Operating voltage	24V	12V	5V	220V	220V	220V
AC/DC	DC	DC	DC	AC	AC	AC
Brand	Prisma SRL	GoodPumps	Atlas scientific	DeLonghi	Blauberg	Solea
Tecnical specifications	142lm/W emission angle > 120°	6l/min	0.5 to 105 ml/min	7000 BTU	600m^3/h	max temp 65 °C

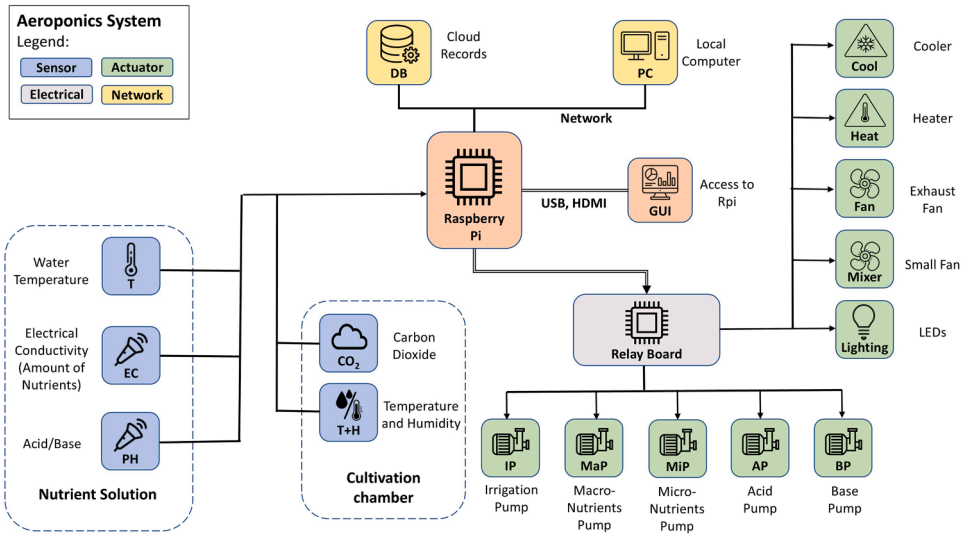


Fig. 1. Schematic representation of the electronic and communication systems.

RPi to manage data acquisition, communication, control logic, and actuator operations. Mycodo also provides a graphical interface for displaying sensor readings, historical data, and system notifications as well as easily constructible functions to modulate each actuator according to time-based commands (e.g. regulate lighting according to the desired photoperiod) or event-based commands (e.g. activate heating according to the temperature readings of the sensor). The actuators were connected via GPIO pins to the RPi and controlled using relays for power switching. This setup ensured precise environmental control, which was crucial for maintaining the nutrient solution EC and pH, and the desired environmental factors. Sensor data were both collected locally and sent to the cloud, using MQTT protocol [25].

2.1.2. Plant material and growing conditions

Seeds of butterhead lettuce (A3036, Gautier semences, FR) were sown in 1 cm^2 rock wool cubes. Cubes were placed in a 228-spot tray and irrigated with tap water, then put in the dark at 22 $°C \pm 2$ °C for 72 h for germination. After the germination, the tray with young sprouts was placed on the aeroponic system described in Section 2.1.1. Each growing cycle lasted three weeks, which was sufficient for cultivating baby lettuce. Moreover, early developmental stages are highly responsive to environmental conditions, resulting in higher variability among different growing conditions [71], which is essential for developing dynamic protocols. Each plant had around 10.5 cm^2 of growth space during the first week. Then, due to plant removal (for analysis), the remaining plants had 21 cm^2 at the beginning of the second week and 80 cm^2 at the third week.

During the whole cultivation period, the photoperiod was kept at 16/8 h of light and dark, respectively [35,63], relative humidity was set at 60 %±5 % (even though actual data showed a variation of 68 % ±12 % among all the growing cycles, due to a slower response of the humidity control system, compared to the temperature control system) [16,35], and CO_2 was at atmospheric level (420–450 ppm) [19,35].

The nutrient solution was prepared by diluting the commercially available Mills nutrient solution: NH_4 0.3 %, NO_3 3.2 %, K_2O 4.3 %, CaO 5.1 %, Fe 0.042 %, P_2O_5 4.3 %, MgO 1.1 %, SO_3 2.2 %, B 0.0045 %, Cu 0.0025 %, Mn 0.02 %, Mo 0.001 %, Zn 0.01 %.

Plant roots were sprayed with the nutrient solution two minutes



Fig. 2. Particular of one of the cultivations chambers.

every 15 min (within the range proposed by [11] and [51].

The pH of the nutrient solution was adjusted with acetic acid or ammonia and kept between 5.5. and 6.5 [35,66], and nutrient solution temperature was measured and found to be at the chamber temperature ± 2 °C.

The DoE experiment was designed considering two levels of temperature, nutrient solution concentration, and light intensity, as detailed in Section 2.2.

2.2. Dynamic protocol design

The dynamic protocol design involves the DoE and the weekly data analysis. First, the DoE is described, and then the selected indicators are listed, including a new indicator (called "growing factor"), which proves itself essential for weekly data analysis. Finally, DoE results are reported and used to define the dynamic protocol specifications.

2.2.1. Design of experiment

We adopted a 2^3 factorial DoE to systematically investigate the effects of three key factors (temperature, nutrient solution concentration, and light intensity) on the productivity of an aeroponically grown lettuce in an indoor SPS.

It is important to note that while nutrient solution concentration, light intensity, and temperature are critical factors influencing productivity [35,63,74], other variables such as pH level [6], humidity [20], and CO_2 concentration [47] may also significantly impact the system's performance, in terms of overall productivity. However, including additional factors in the analysis would exponentially increase the number of required experimental runs due to the nature of factorial designs. Therefore, this study focuses on the three selected factors to provide a manageable yet comprehensive analysis of their effects on system productivity, whereas the other environmental factors were kept fixed at given values, as specified further in this section. Specifically, CO_2 concentration varied between 420 and 450 ppm on a daily basis, whereas chamber humidity fluctuated between 56 % and 80 % across all growing cycles. Even though our control system had a smaller range (60 %±5 %). Finally, the nutrient solution pH was maintained around 6, with experimental data showing a minimum of 5.5 and a maximum of 6.5. Each factor was examined at two levels. According to [35] and [76], nutrient levels were settled at 1000±50 μS/cm (low, N-) and 1600±50 μS/cm (high, $N+$), whereas the chosen values for lighting were 150±20 μmol/m^2s (low, L-) and 250±20 μmol/m^2s (high, $L+$) [63,79]. Considering the chosen photoperiod of 16/8 h of light/dark, these values correspond respectively to a daily light integral (DLI) of 8.64±1.15 mol/m^2d and 14.4 ± 1.15 mol/m^2d. Regarding temperature, the low value (T-) was set at 20 $°C \pm 1$ and the high one ($T+$) at 25±1 °C [16,4]. Values are summarized in Table 2.

The eight combinations are indicated as ($T+$, N-, L-), (T-, $N+$, $L+$), (T-, N-, L-), (T-, $N+$, L-), (T-, N-, $L+$), ($T+$, $N+$, L-), ($T+$, $N+$, $L+$), ($T+$, N-, $L+$).

The experiment employed a randomized design to assign the eight treatment combinations to experimental units, thereby mitigating potential biases and ensuring the robustness of the findings. Each treatment was replicated once, for a total of 16 growing cycles, to enhance the reliability and reproducibility of the results. For each growing cycle and time point, six plants were collected and analyzed; since each experiment was replicated once, we had 12 observations per growing condition and time point. This number of observations was chosen with the aim of achieving a statistical power of 80 % with 5 % confidence level and an effect size of 40 %, as computed using G*Power statistical software [34]. By systematically varying temperature, nutrient solution concentration, and light intensity, we can comprehensively assess their individual and combined effects on system production capability, in terms of biomass accumulation.

2.2.2. Lettuce growth indicators identification and collection

The following indicators are widely used to assess crop productivity because of their ease of measure combined with high reliability [32]: Fresh weight (FW), Dry weight (DW), and Leaf area (LA).

Additionally, the nitrate content is considered a good indicator for assessing crop quality because it reflects plants' ability to absorb nutrients. However, it also is a dangerous molecule for human health. Indeed, high nitrate consumption has been linked to conditions such as methemoglobinemia and potential carcinogens [7]. Therefore, its concentration must be under a certain low-defined level ([15].). European legislation sets both the Acceptable Daily Intake (ADI) for nitrate [72] and the limits of its concentration in vegetables [73]. The first is set at 3.7 mg/kg of body weight, whereas the second oscillates between 2000

Table 2
Summary of DoE tested parameters.

Factor	Symbol	Low level	High level
Temperature	T	20 $°C \pm 1$ °C	25 $°C \pm 1$ °C
Nutrient solution concentration	N	1000±50 μS/cm	1600±50 μS/cm
Light intensity	L	150±20 μmol/m^2s	250±20 μmol/m^2s
Daily light integral (DLI)		8.64±1.15 mol/m^2d	14.41.15 mol/m^2d

and 5000 mg NO_3/kg of lettuce, depending on the period of the year and on the cultivation technique. These values may result in exceeding the ADI by almost 13 % by consuming 50 g of high-nitrate lettuce by a 60 kg person. Thus overtaking the safe level and entering the dangerous threshold. Generally, lettuce is unlikely to exceed 4000 mg NO_3/kg FW, but there is a strong correlation between nitrate concentrations and lettuce growing techniques [65,37].

To collect the above indicators, the following procedure has been done weekly during the three-week-long cultivation period. Fig. 3 shows an example of weekly lettuce samples. The cultivation area was divided into three sub-sections (or sectors) based on a geometrical basis. A total of six plants (two per sector) were randomly chosen from the available ones and cut at the root-crown level, then immediately weighted for FW data collection (Radwag PS1000.R2). Then, every leaf for each plant was scanned (Petiole Pro APP, ([58].)) for total LA acquisition. After that plants were put into a dehydrator at 68 °C for 48 h, then weighed for DW. Nitrate content analysis was conducted as follows: at least 3 g of lettuce was collected per sector (ensuring three measures per condition per week) and frozen for analysis, for a maximum of four weeks. Nitrate analysis was settled following BS EN 12014–2:2017 ([26].). The total sample collected was homogenized with a mixer, and then around 1 g was weighed for analysis. The weighted sample was transferred to a 100 ml beaker with circa 40 ml of ultrapure (MilliQ) water at 80 °C, then brought to boiling point and boiled for 15 min. After this process, the solution was kept at room temperature until cooled down, then filtered through a 25 μm filter (Whatman filter grade 4) and collected in a 50 ml volumetric flask (class A, Vitlab GmbH, DE). 200 μl of the solution were collected and purified using nitrate kit (LCK340, Hach, DE), then analyzed at 220 nm in a spectrophotometer (Hach, DE).

In addition to fresh weight, dry weight, and leaf area, we defined a new indicator named Growing Factor (GF). GF is based on FW data, and it is defined as the ratio between the crop FW at a given time t and the crop FW at time t-1, as reported in the following formula:

$$GF(t) = FW(t)/FW(t-1).$$

Given the collection of the indicator FW being a destructive measure, the value of GF is obtained as a mean of the ratio between every measure obtained at time t and every measure obtained at time t-1. This GF calculation has a limitation since FW(t) and FW(t-1) data do not belong to the same plant. Therefore, we analyse the statistical power of the obtained data (using G*Power [34]) and find that it has a statistical power of 95 %, with a 5 % confidence level and an effect size of 20 %. Nevertheless, GF suffers from cross-plant variability and might be improved using a non-destructive type of measurement. Moreover, all the obtained data were checked for outliers using the methodology proposed by [46], and the data that fulfilled the definition was eliminated.

Table 3 summarizes the data collected. Particularly, it shows the mean and standard deviation of FW, DW, LA, and nitrate content of lettuce plants harvested at three different time points (i.e., after one, two, and three weeks of growth) for each combination of tested environmental factors (T, N, L). It also reports the GF, which is computed only for the second and third cycles of lettuce growth.

Table 3
Overview of the data collection of FW, DW, LA, Nitrate content, and GF, divided by time point and growing conditions (T-: 20 °C; *T*+: 25 °C; N-: 1000 μS/cm; *N*+: 1600 μS/cm; l-: 150 μmol/m^{2}s; *L*+: 250 μmol/m^{2}s).

Time Point	Condition	Fresh Weight (mg)	Dry Weight (mg)	Leaf Area (cm^2)	Nitrate Content (mg/ Kg_{FW})	Growing Factor
1	*T* + *N*-L-	114.8 ± 19.8	5.2 ± 2.1	7.1 ± 1.3	704.5 ± 72.1	NA
1	T-*N* + *L*+	87.5 ± 16.3	4.7 ± 2.1	4.4 ± 0.7	447.7 ± 96.4	NA
1	T-N-L-	77.1 ± 21.2	2.5 ± 1.1	4.0 ± 1.4	634.0 ± 241.7	NA
1	T-*N* + *L*-	83.5 ± 13.6	4.8 ± 1.0	4.1 ± 1.2	709.3 ± 78.4	NA
1	T-N-*L*+	109.7 ± 16.7	5.9 ± 1.8	4.5 ± 0.8	439.9 ± 112.8	NA
1	*T* + *N* + *L*-	113.8 ± 16.7	4.5 ± 2.2	6.6 ± 0.9	704.5 ± 67.0	NA
1	*T* + *N* + *L*+	189.8 ± 45.8	12.5 ± 4.4	11.1 ± 2.6	563.2 ± 39.3	NA
1	*T* + *N*-*L*+	179.0 ± 33.4	10.6 ± 3.9	8.5 ± 1.2	548.0 ± 49.2	NA
2	*T* + *N*-L-	1482.8 ± 506.4	81.0 ± 28.3	84.0 ± 26.4	812.9 ± 216.1	12.8 ± 3.0
2	T-*N* + *L*+	1285.2 ± 227.2	75.2 ± 16.2	56.2 ± 9.3	868.4 ± 444.2	14.8 ± 2.6
2	T-N-L-	877.8 ± 214.8	49.4 ± 13.0	45.5 ± 12.3	972.7 ± 450.3	11.7 ± 2.2
2	T-*N* + *L*-	1014.1 ± 181.5	53.6 ± 12.1	51.4 ± 8.7	764.4 ± 131.1	12.3 ± 2.4
2	T-N-*L*+	1310.8 ± 215.9	79.8 ± 14.7	57.3 ± 8.8	651.6 ± 153.7	12.1 ± 2.5
2	*T* + *N* + *L*-	1534.1 ± 358.1	81.9 ± 25.2	80.9 ± 17.8	806.5 ± 113.7	13.6 ± 2.8
2	*T* + *N* + *L*+	3340.8 ± 767.9	181.3 ± 45.0	132.7 ± 18.0	841.1 ± 155.1	17.7 ± 1.9
2	*T* + *N*-*L*+	2744 ±769.0	157.3 ± 46.0	130.6 ± 33.7	781.0 ± 165.3	15.4 ± 3.2
3	*T* + *N*-L-	8518.2 ± 2371.7	385.2 ± 90.8	391.8 ± 87.8	806.1 ± 164.5	5.9 ± 1.5
3	T-*N* + *L*+	7907.8 ± 1507.7	411.6 ± 78.9	274.8 ± 45.7	968.7 ± 422.7	6.2 ± 1.2
3	T-N-L-	5494.2 ± 2186.1	284.9 ± 112.8	228.7 ± 96.8	921.4 ± 372.8	6.1 ± 0.9
3	T-*N* + *L*-	5818.0 ± 1344.9	272.4 ± 60.9	245.1 ± 49.6	896.5 ± 56.0	5.8 ± 1.3
3	T-N-*L*+	8523.7 ± 1577.5	452.8 ± 102.1	313.8 ± 50.5	480.6 ± 156.0	6.6 ± 1.2
3	*T* + *N* + *L*-	7886.3 ± 1206.1	415.8 ± 110.4	375.3 ± 73.4	903.5 ± 447.9	5.2 ± 0.9
3	*T* + *N* + *L*+	16,881.3 ± 6039.8	712.4 ± 241.5	519.0 ± 119.0	723.3 ± 85.9	5.0 ± 0.7
3	*T* + *N*-*L*+	15,945.0 ± 3393.1	716.2 ± 113.2	583.6 ± 91.7	634.9 ± 202.6	6.0 ± 1.2



Fig. 3. Lettuce plants in the three harvesting points, after one, two, and three weeks of growth.

The collected data were analyzed with the Minitab 21 software [24]. Firstly, we performed a correlation analysis among the variables, by computing the Pearson correlation coefficient [70]. We found that FW has a high correlation with both LA (0.973) and DW (0.987). Therefore, we decided to leave LA and DW out of the analysis and focus only on FW, nitrate content, and GF.

2.2.3. Data analysis: fresh weight

As expected, FW values vary significantly under different conditions; for instance, after one week of growth, FW averaged between a minimum of 77,1 mg (with T, N, and L at the low levels) and a maximum of 190 mg (with all the tested variables at high levels). The second and

third weeks show a similar trend. However, during the third week, differences between different conditions became less evident than in the first two weeks.

Table 4 summarizes the analysis of variance regarding single and multiple effects on FW of T, N, and L. P-values scored lower than 0.001 on both L and T regardless of the harvest week, but they had a higher value regarding the effect of N. Consequently, also the combined effect (N*T, N*L, and N*L*T) had no statistical significance on the FW.

Fig. 4 provides a graphical view of the crop's FW, according to both the growing condition and the time points. Data highlights the importance of both light (L) and temperature (T) on the FW at each harvest point, whereas the nutrient solution concentration (N) seemed to have little influence on it. From Fig. 4, it is also possible to appreciate the effect of the growing condition on the crop growth rate: for instance, the second condition (T-*N* + *L*+) appears to have a higher growth rate during the second week compared to the first condition (*T* + *N*-L-), whereas the behavior was opposite on the first week. Also, condition seven (*T* + *N* + *L*-) and eight (*T* + *N*-*L*+) have the same growth rate at week 1, then the first grows faster during the second week and slower during the third week. This aspect of crop growth is better explained by the analysis of the GF, in Section 2.2.5.

2.2.4. Data analysis: nitrate content

Data showed that nitrate levels in leaves are mostly dependent on the light conditions and temperature rather than on the nutritional level. Particularly, light affects nitrate content in the first and third weeks and temperature in the first and second weeks, as shown in Table 5.

Fig. 5 reports the average nitrate levels per week and light treatment, showing a common trend: higher light levels corresponding to a lower nitrate content. As nitrate is a pivotal element in plant metabolism [22], it is unsurprising that high light levels pair with low nitrate concentration in leaves. Indeed, high light corresponds to high photosynthetic activity, which generally speeds up plant metabolism, making it possible to use the available nitrate in molecular biosynthesis. On the other hand, low light means low metabolic activity, so the nitrate remains stored within leaves cells.

Generally, all the nitrate levels measured in this experiment remained below the low-permitted concentration ranges [50]. Moreover, we showed that by using the conditions that increase lettuce production, we permit a high rate of nitrate assimilation, resulting in a final product that is safer for human consumption.

2.2.5. Data analysis: growing factor

GF analysis reveals a broader distribution of responses to the environmental factors of growth concerning the FW analysis, especially at the end of the second week. Indeed, GF values are larger at the second time point rather than in the third one, suggesting the importance of this first period of lettuce growth to increase production at the end of the cycle. In the first point (week 2) GF scores between 11.7 and 17.7 whereas in the following one, the growth rate decreases, with the GF scoring between 5.0 and 6.6, as reported in Table 3.

Interestingly, the condition that provides the maximum FW does not correspond to the ones that score the higher GF, by time point.

Table 4
Summary of the key statistics of ANOVA analysis on FW.

Environmental factors	Harvest week 1		Harvest week 2		Harvest week 3	
	F-value	P-value	F-value	P-value	F-value	P-value
T	135.73	0.000	146.43	0.000	83.41	0.000
N	0.09	0.771	3.98	0.049	0.00	0.996
L	74.00	0.000	97.85	0.000	83.83	0.000
T*N	1.54	0.218	2.00	0.161	0.06	0.800
T*L	25.43	0.000	38.40	0.000	23.08	0.000
L*N	0.67	0.415	1.02	0.315	0.07	0.790
T*N*L	3.85	0.053	3.46	0.066	1.14	0.289

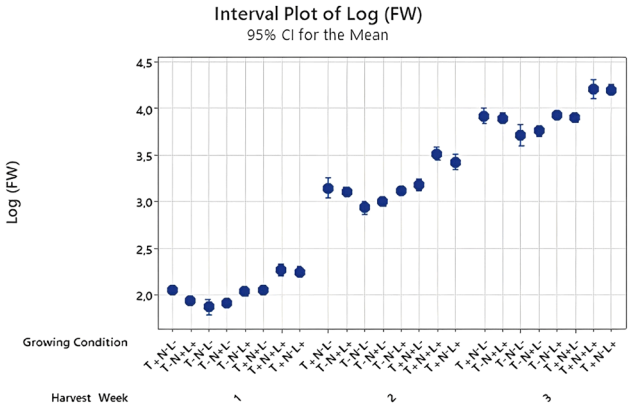


Fig. 4. Interval plot of the FW (expressed in logarithmic scale) by growing condition and time point. (T-: 20 °C; *T*+: 25 °C; N-: 1000 µS/cm; *N*+: 1600 µS/cm; L-: 150 µmol/m^{2}s; *L*+: 250 µmol/m^{2}s).

Table 5
Summary of the key statistics of ANOVA analysis on Nitrate Content.

Environmental factors	Harvest week 1		Harvest week 2		Harvest week 3	
	F-value	P-value	F-value	P-value	F-value	P-value
T	15.90	0.000	13.80	0.001	2.09	0.158
N	6.14	0.018	0.20	0.654	4.16	0.049
L	40.13	0.000	0.78	0.383	6.83	0.013
T*N	4.69	0.038	0.00	0.947	0.53	0.470
T*L	0.03	0.864	0.83	0.369	0.02	0.893
L*N	2.36	0.134	2.01	0.166	0.29	0.591
T*N*L	3.42	0.073	4.24	0.047	0.23	0.635

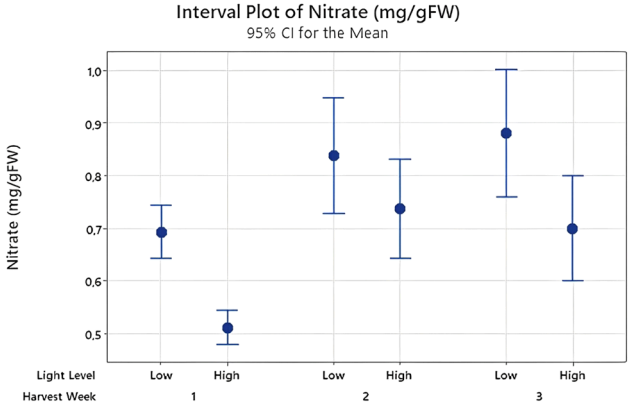


Fig. 5. Average nitrate content in plant leaf according to the level of light exposure and the harvest week.

Moreover, the condition that has the higher GF in the second time point (*T*+, *N*+, *L*+), has the lower one in the third time point.

This suggests that a combination of growing conditions may lead to a higher overall productivity of the system.

The analysis of the GF yielded unexpected results. During the second week of growth, the highest GF is obtained at the same condition that provides the higher FW (*T*+, *N*+, *L*+), but this effect appears to be due to all the tested variables (with p values below 0.001 for each of them), while in the FW analysis, N seemed to have almost no effect. Moreover, N has a higher effect than both T and L on the GF, as shown in Table 6. During the third week of growth, the effects of the environmental factors on the GF change considerably. Indeed, the main effect analysis undoubtedly reveals that the highest impact on the GF value is given by N, followed by L, and that T has almost no effect (as shown by the F-values

Table 6
Summary of the key statistics of ANOVA analysis on GF.

Environmental factors	Harvest week 2		Harvest week 3	
	F-value	P-value	F-value	P-value
T	25.06	0.000	0.19	0.659
N	115.06	0.000	74.39	0.000
L	41.72	0.000	28.77	0.000
T*N	12.34	0.000	42.67	0.000
T*L	0.01	0.904	1.79	0.182
L*N	58.44	0.000	21.75	0.000
T*N*L	11.36	0.001	21.17	0.000

in Table 6). Moreover, differently from what was observed in the first week, the low levels of N and T tend to increase the GF value (as shown in Table 3).

The apparent contradiction regarding the effect of N on FW and GF can be explained by considering the different meanings of the two indicators: FW accounts for everything that happened to the crop from germination to a given harvest point, and this implies that the effect of N at the third-week accounts for the previous 21 days of growth. On the other hand, GF only accounts for what happened in the previous 7 days, per each time point. This means that the combination of the period of negative effect of N, followed by a period of positive effect, will produce a "no effect result" on FW, but not on the GF.

All these considerations were used to develop the dynamic protocol, as further detailed in Section 3.

3. Results

For defining the dynamic protocol, we use the following procedure: for the first week, we chose the condition that gave the highest FW, and for the second and third week we used the conditions that obtained the highest GF. Therefore, the dynamic protocol used the condition $T+N+L+$ for the first two weeks and the condition T-N-$L+$ for the third one.

To test the validity of the approach, we set such conditions in the cultivation chamber and collect the corresponding data for the two replicas. Table 7 reports the obtained data, and Fig. 6 shows the FW obtained with the dynamic protocol (red points) in comparison with the previous data.

As expected during the first two weeks, the dynamic conditions show a growth very similar to the condition $T+N+L+$, since they are each other's replicas. After the conditions change on day 14, the growth rate of the two diverges, and the dynamically grown lettuce increases its weight, overtaking the best-fixed condition.

Overall, the dynamic protocol brings an average increase in productivity of 24.89 %, at the end of the three-week long growing period.

About the quality parameter nitrate content, substantial changes are not observable. As reported in Section 2.2.4, nitrate levels seem to correlate with light or temperature depending on the harvest week, rather than with nutrient levels. Since the dynamic protocol uses high light levels, no significant variations are highlighted, as reported in Table 7. Consequently, the variation in the growing conditions that permitted an increment of the biomass accumulation does not change the quality of the crop.

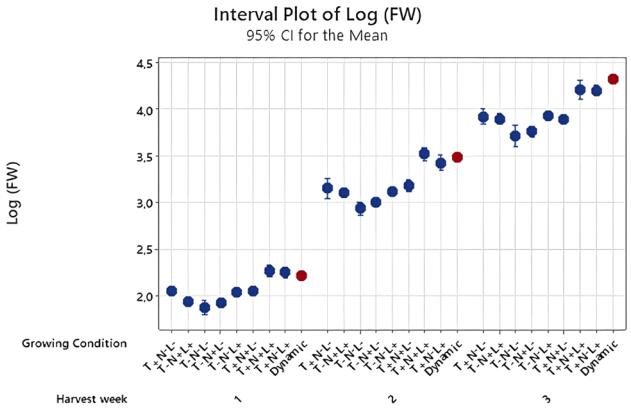


Fig. 6. Interval plot of the FW (expressed in logarithmic scale) by growing condition and time point, dynamic protocol results are highlighted in red. (T-: 20 °C; $T+$: 25 °C; N-: 1000 µS/cm; $N+$: 1600 µS/cm; L-: 150 µmol/m^{2}s; $L+$: 250 µmol/m^{2}s).

4. Discussion

In this work, we analyzed, using a DoE approach, the effects of three climatic variables (temperature - T, nutrient solution concentration - N, and light intensity - L) on the biomass accumulation (expressed as FW, DW and LA) and nitrate content of aeroponically grown lettuce plants. Even though DW and LA provide valuable information on plant health and growth, for the sake of conciseness, we decided to focus mainly on FW, since DW and LA have a significant correlation with FW and do not provide additional information in the context of our work. FW reflects crops' capability to respond to different environmental factors. For instance, lighting has a direct effect on plant photosynthesis rate since it provides the energy necessary to fix carbon dioxide [80]. Temperature and CO_2 concentration may influence Rubisco's efficiency [59,18] and, therefore, the carbon fixation rate. Nutrient solution concentration can lead to improper development if it is too low [1], while the accumulation of specific nutrients can be toxic to the plant [44]. Nutrient solution pH, on the other hand, has an effect both on nutrient availability [62] and root health [42]. Humidity directly relates to stomatal apertures and, therefore, to internal CO_2 concentrations [52].

DoE allowed us to investigate the effect of the system's input variables on the output, which represents the marketable part of the crop.

We aimed to increase the system's productivity by modifying the environmental factors at different time points rather than using more resources (e.g., increasing light intensity).

DoE results showed a high effect of T and L on FW, while N seemed to have almost no effect on it. The FW indicator refers to the status of the plant at a given time point of analysis; for example, FW obtained in the third week is the final result of the conditions the crop had from the whole growing period, this means that DoE analysis conducted on it reveals the effect of T, N, and L on it as the average effect along the whole 3-weeks long growing period. To investigate the effect of the input variable between two time points, we introduced the GF indicator. GF considers the effect of environmental factors only in the period between two consecutive time points. Here, we defined GF concerning the FW, but it can be computed using other productivity indicators, such as DW or LA. The same approach can be used to interpolate indicators of

Table 7
Overview of the dynamic protocol data collection of FW, DW, LA, Nitrate content, and GF, divided by harvest time point and growing conditions (T-: 20 °C; $T+$: 25 °C; N-: 1000 µS/cm; $N+$: 1600 µS/cm; L-: 150 µmol/m^{2}s; $L+$: 250 µmol/m^{2}s).

Time Point	Condition	Mean$_{FW}$ (mg)	Mean$_{DW}$ (mg)	Mean$_{LA}$ (cm^2)	Nitrate content (mg/Kg$_{FW}$)	Growing Factor
1	$T+N+L+$	165.5 ± 23.4	11.7 ± 3.8	10.0 ± 1.5	536.7 ± 35.5	NA
2	$T+N+L+$	3005.9 ± 187.5	171.7 ± 26.6	136.4 ± 10.3	548.5 ± 96.8	18.2 ± 2.2
3	T-N-$L+$	21,083.1 ± 3282.0	1103.2 ± 159.1	656.4 ± 69.6	638.1 ± 110.0	7.0 ± 1.1

other dimensions (e.g. quality). Since the nitrate level appeared not to correlate with the time of growth, we did not develop an indicator to describe the evolution of the nitrate over time. GF has an intrinsic limitation since it is computed using data from two different time points that do not belong to the same plant, which is destroyed during destructive measurements. We attempted to mitigate this effect by computing up to 72 measurements per point and by observing that this sample size guarantees high statistical power and, therefore, GF robustness. GF can also be computed using non-destructive analysis (e.g. imaging estimations) on the same plants; this alternative may be more precise because data would come from the same plant. However, on the other hand, destructive measures are more precise in measuring FW or LA.

Interestingly, we identified a shallow effect of N when we analyzed the effect on FW in the second and third week, whereas the analysis on the GF revealed that the small effect was an average of the strong positive effect during the second week and the strong negative effect during the third week. This result aligns with [3], where lettuce reduced need for nutrients was reported, comparing the third to the second weeks of growth. According to our observations, lettuce benefits from a lower nutrient concentration during the third week of growth, which is closer to its physiological needs.

Besides the effect of T, N and L on plant growth, other climatic parameters can have a strong effect, these include nutrient solution pH, humidity and CO_2 concentration. The first two were controlled and kept as costant as possible, even though small fluctuations were observed in the whole 7-month-long experimental period, whereas CO_2 was only monitored, but found to have only small daily based fluctuations. Although these fluctuations may have influenced reported results, they are mitigated by the experimental replica.

The reported findings allowed us to answer our first research question. Indeed, crops respond differently to the same environmental factors at different time points of their growth. Using the GF, we identified optimal growing conditions (in terms of biomass production) that vary according to the week of growth. Specifically, plants grew faster at the condition $T + N + L+$ during the first two weeks of growth, but this was no longer the case during the third week.

Therefore, GF is essential for developing a dynamic growing protocol because of its ability to describe crop growth in specific time intervals. Indeed, GF highlighted a strong correlation between nutrition and growing rate (with p-values lower than 0.001) which was hidden in other indiatrors (e.g. FW or DW). This correlation was pivotal in develping the dynamic protocol resulting in an increased system productivity.

DoE results were then used to define a dynamic protocol, which does not use fixed conditions for the growing period, but changes them, selecting the most favorable one, week by week. Our research demonstrates that the development of dynamic growing protocols, achieved through a reasonable number of experiments using a DoE experimental construction and the development of suitable indicators, can significantly enhance system performances. Indeed, we observed a potential increase in production of almost 25 % without increasing resource consumption.

This achievement answers our second research question: dynamic protocols can indeed increase crop production better than uniform environmental factors.

These protocols can be tailored to the desired outcome, whether it is increasing production or saving resources. By selecting diverse output parameters (e.g. water or energy consumption), a protocol could be developed to maintain a certain production level while conserving resources.

Our results apply specifically to the tested lettuce cultivar and cannot be directly applied to other plant species. Nevertheless, the described methodology could be applied to obtain dynamic protocols for other crops. Moreover, our findings help reduce experimental needs since similar crops may behave similarly, for example, by having a minimal response to nutrient solution concentration. The removal of one of the tested variables can halve the number of experiments. At this point in the research, it is not possible to identify whether and which other crops may benefit from a dynamic growing protocol; however, it is reasonable to speculate that other crops may also benefit from dynamic growing conditions. Furthermore, future research in this field could explore the results of shorter time points. This approach could significantly increase the resolution of the results, allowing for a thinner adjustment of the growing conditions which in turn can better meet the plants' evolving needs. Moreover, doubts remain regarding the potential advantages of this approach over longer cultivation times (e.g., up to 5–7 weeks of growth), which could be addressed through future research.

5. Conclusion

In this study, we evaluated the effects of environmental parameters - temperature, nutrient solution concentration, and light intensity - on the growth and quality of lettuce in an SPS.. We collected three important indicators of crop productivity (DW, FW, and LA) that provide important metrics about crop productivity and health. Although their combined importance is significant, these indicators are not relevant for developing a dynamic protocol; therefore, we relied solely on FW. Our findings confirm the potential of dynamic control protocols to enhance productivity in SPSs by adjusting growing conditions at different time points rather than maintaining fixed conditions throughout the crop cycle. The dynamic protocol we developed demonstrated an average productivity increase of nearly 25 % at the end of the growing cycle, compared to fixed condition protocols, without increasing resource use. This result highlights the importance of accounting for plants' evolving needs during the growth cycle. Additionally, our analysis of nitrate content confirmed that crop quality remains consistent, unaffected by the dynamic changes in growing conditions, thus ensuring the safety and marketability of the product. Indeed, under all the tested conditions, nitrate concentration remained far beneath the law level, allowing for safe consumption of the product.

The GF indicator showed how environmental factors impact growth differently at each stage. This suggests that future systems should increasingly focus on fine-tuning input conditions. Introducing such dynamic systems presents a new frontier for sustainable, resource-efficient farming, particularly in SPSs, where maximizing input efficiency is crucial.

Our research not only emphasizes the effectiveness of optimizing environmental factors dynamically but also introduces a method for developing dynamic protocols. This method can potentially make more species suitable for indoor farming, opening new possibilities for the future of this agricultural method.

Although the present work does not include a cost-benefit analysis, which is pivotal for improving the economic viability of VFPSs, we believe that increasing the productivity of this kind of systems without increasing their technological complexity or resource needs might be the first step toward lowering production costs. Indeed, there were no differences in the complexity of the control architecture or maintenance-labour demands between the two approaches. Future studies could address the economic aspect of this production technique, and they can further refine this approach by exploring shorter time points (up to real-time monitoring of plant development) as well as additional indicators to fine-tune growth conditions, leading to even more efficient and sustainable production practices in SPSs.

Supplementary material

Data collected in this experiment is available for researchers under reasonable request to the authors.

Ethics statement

Not applicable: This manuscript does not include human or animal research.

CRediT authorship contribution statement

Nicolò Grasso: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benedetta Fasciolo:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Giulia Bruno:** Writing – review & editing, Validation, Supervision, Project administration, Methodology. **Paolo Chiabert:** Supervision.

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.

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Data availability

Data will be made available on request.

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