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Article

A Rotational Cultivation System for Indoor-Grown Lettuces: Feasibility in Terms of Yields, Resource Efficiencies, Quality, and Postharvest Storage Capacity

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Abstract: Indoor farming in Plant Factories with Artificial Lighting (PFAL) offers optimized growing conditions and higher water, light, and land surface use efficiencies compared to greenhouses or open field agriculture but faces challenges related to energy consumption. The objective of this work is to evaluate the feasibility of using a rotational cultivation system for indoor-grown lettuce production. We compared a rotational cultivation system to a horizontal control cultivation system in terms of yields, resource efficiencies, quality at harvest, and postharvest storage capacity. No significant differences were observed in yields, WUE, LUE, or postharvest storage capacity between the systems. Energy and Land Surface Use Efficiencies were higher in the rotational cultivation system compared to the control, and consistent with the literature. However, a slight trend toward lower fresh and dry weights throughout the cultivation period in the rotational system was noted, correlating with reduced net photosynthesis during the first two hours and at the end of the lighting period. This effect was attributed to decreased stomatal conductance and photosystem II efficiency. Furthermore, the rotational cultivation system modified the quality by modifying the global polyphenol profile of lettuces compared to the control. Based on yields and efficiencies, we showed the feasibility of using a rotational cultivation system for indoor lettuce production.

Keywords: controlled environment agriculture; resource efficiency; growth; photosynthesis; gravity perception

1. Introduction

Indoor farming is an alternative method of agriculture, complementary with conventional agriculture to face the challenges of feeding a constantly growing and increasingly urbanized population in the context of global warming [1,2]. Indoor farming occurs in Plant Factories with Artificial Lighting (PFAL), in which climatic parameters are controlled, and the light is provided by lamps, *i.e.* independent from natural sunlight. Those conditions are optimal for plants growth, which explains that indoor grown plants have higher Light, Water and Land Surface Use Efficiencies (LUE, WUE, LSUE, respectively) than greenhouses or open field productions [3–5]. However, the main

challenge of indoor farming is energy consumption. For example, it has been presented that an hydroponic indoor production needs eighty-two times more $\text{kJ kg}^{-1} \text{an}^{-1}$ than a production in conventional agriculture [6]. The cost of indoor production is three to five times higher than open field [7] and, at an industrial scale, electricity costs represents 25% of the total cost of lettuce produced indoor [8,9]. Among parameters, lighting plays a key role in energy consumption, as it represents from 50 to 80% of the total electricity consumption of an PFAL [4,8,10,11].

It seems challenging to reduce energy consumption indoor farming by reducing the lighting without compromising yields. Indeed, lettuce dry weight correlates with the Daily Light Integral (DLI) in the range of 250 to 650 $\mu\text{moles photons m}^{-2}$ [12–15]. Moreover, extensive research has already optimized lighting parameters, with optimal light quality between 400-700 nm, far-red supplementation (700-800 nm), optimal light intensity of 250 $\mu\text{moles photons m}^{-2} \text{s}^{-1}$ intensity, and an optimal photoperiod of 16 hours resulting in 14.4 moles of photons $\text{m}^{-2} \text{d}^{-1}$ [14,16–19]. Another way to reduce energy consumption could be to decrease the number of lamps used, while keeping other lighting parameters constant. In standard horizontal cultivation system, this is challenging in terms of light homogeneity. However, light homogeneity is a key parameter in indoor farming as one of its advantages is to provide consistent, uniform, and stable production throughout the year.

A promising approach to reduce energy consumption, without compromising yields or lighting homogeneity, is through new cultivation systems. Rotational cultivation systems require a low number of lamps per plant while maintaining perfect light homogeneity among them. Specifically, a small number of lamps emitting light at 360°C can be positioned at the center of the cylinder, with plants rotating around the lamps, ensuring that each plant remains equidistant from the light source.

To our knowledge, only one recent study focused on the feasibility of indoor productions in a rotational cultivation system [20]. The objective of this work is thus to compare lettuces grown in a rotational cultivation system and in a horizontal control one in terms of yields, Water Use, Light Use, Energy Use and Land Surface Use Efficiencies, as well as quality at harvest and postharvest storage capacity. The GiGrow® rotational cultivation system has been studied, as it was designed for industrial-scale use. The rotational cultivation system used rotates at a speed of 50 minutes per revolution, which induces a constant perturbation of plants gravity perception. Thus, to bring insight into the rotation on plants physiology, we also studied photosynthesis. The plant matrix used was *L. sativa* and is among the most frequently consumed vegetable of nutritional interest globally [21], and is a standard production of indoor farming facilities.

2. Materials and Methods

2.1. Plant Material and Germination

Butterhead lettuce (*Lactuca sativa* L. var. fairly, 'Enza Zaden', The Netherlands) seeds were sown in 144 (16x9) holes germination trays filled with potting soil (TBSP, 'Florentaise', France). The photoperiod was 16/8 (16 hours of light, 8 hours of darkness) with respective temperatures of 24/19°C and relative humidity of 65%. Water was provided until saturation by sub irrigation on a 3-days basis with a germination solution composed of 3,2, 0,6, 0,9, 1,3, 0,8 and 0,1 mmol L^{-1} of nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, respectively. The electroconductivity (EC) of the irrigation solution was equal to 0,5 mS cm^{-1} with a pH of 5,6. Light intensity was set at 130 $\mu\text{moles m}^{-2} \text{s}^{-1}$ with Red:Blue 3:1 and $\approx 17\%$ of white light, provided by LED lamps (T10 LED Grow Tube Light, HW-GL-T10-1200-36W-3Y, China).

2.2. Plant Growth

After 14 days of germination, plants were transplanted in two different cultivation systems:

- A control cultivation system, horizontal (Figure 1A). Lettuces grown in this cultivation system are referred to as CT lettuces. cultivation frames (140 x 210 cm) were installed in a growth chamber and equipped with a drip irrigation system and white light LEDs lamps (LO250

Floodlight, LO250-PSHW06040, 'Vegeled', Belgium). Lettuces were cultivated on pots (2 113 cm³) filled with potting soil (VER4, 'Florentaise', France) with a density of 30 plants m⁻².

- A Gigrow® rotational cultivation system from Futura Gaïa Technology (Figure 1B). Lettuces grown in this cultivation system are referred to as ROT lettuces. Lettuces were transplanted in 5 holes stainless steel trays (72 x 15 x 4,5 ; l x w x h, in cm) filled with potting soil (VER4, 'Florentaise', France), with a density of 30 plants m⁻². Plants were rotating according to a horizontal rotation axis at a speed of 50 minutes per revolution, which provides a centrifugal force of 1,33x10⁻⁵ Newton, considered neglectable. Light was provided using ceramic metal-halide lamps with a 4200K color temperature (630W double ended, 'Lumatek Ltd', Malta).



Figure 1. Representation of the control cultivation system (A) (CT lettuces) and the rotational cultivation system Gigrow® from Futura Gaïa Technology (B) (ROT lettuces). In the rotational cultivation system, plants were grown in stainless steel trays containing potting soil similar to the control.

In both cultivation systems, the light intensity was 370 ± 20 $\mu\text{moles of photons m}^{-2} \text{ s}^{-1}$, with a Red:Green:Blue ratio of 1:0,8:0,4. Temperature was equal to $24 \pm 0,1^\circ\text{C}$ during the lighting periods and $18,5 \pm 0,5^\circ\text{C}$ during the darkness period in both cultivation systems. Vapor Pressure Deficit were equal to $1 \pm 0,1$ and $0,6 \pm 0,1$ during the lighting and darkness periods, respectively, in both cultivation systems. Irrigation solutions were made with Nutrimix™ of Futura Gaïa Technologies. Water supply was managed to saturate the soil, to avoid water deficiency effects ($2,5 \text{ L plant}^{-1}$ for 31 days). The fertilization solution was composed of 29,3, 1,8, 12,5, 10, 7,5 mmol L⁻¹ of nitrogen, phosphorus, potassium, calcium, and magnesium respectively with an electroconductivity (EC) of 4 mS cm^{-1} and a pH of 6,5. Cultivation time was set for 31 days.

2.3. Measurement and Calculation Methods of Agronomic Parameters

Fresh weight, dry weight and leaf area were studied after 0, 8, 14, 19, 24, 28 and 31 days after transplantation (DAT). Whole plants were harvested, weighed, and all leaves were arranged on a blank surface to take a picture. Leaf area was determined using ImageJ [22]. The dry weight of the whole plants was determined by placing at least two representative leaves in an oven at 80°C for seven days.

Yields and efficiencies were calculated based on fresh and dry weights at harvest, after 31 DAT. Fresh and dry yields were calculated in kilograms of fresh or dry weight, respectively, per square meter. Efficiencies were calculated according to [23] as follows. Light Use Efficiencies (LUE) were calculated in grams of fresh or dry weight per mole of photons by summing the Daily Light Integral (DLI) of each day of cultivation. Water Use Efficiencies (WUE) were calculated in grams of fresh or dry weight per liter of irrigation solution provided throughout the cultivation. Energy Use Efficiencies (EUE) were calculated in milligrams of fresh or dry weight per kilowatt consumed. For the control cultivation system, kilowatt consumption was measured using an electricity meter (CONTAX D TRI 80A LCD, MCI, France). For the rotational cultivation system, kilowatt consumption was also measured using an electricity meter (PowerTag Vigi DT40/iC60 3P+N 63A, Schneider Electric, France). For one day of cultivation, the control cultivation system consumed 203 kWh and the rotational cultivation system consumed 43 kWh of electricity. Land Surface Use Efficiencies (LSUE) were calculated in grams of fresh or dry weight per square meter of land surface used by the cultivation system and per day. For the control, the land surface used was equal to the

cultivation area, while for the rotational cultivation, a production area of 8 m² can fit into a land surface of 2,8 m².

2.4. Glucose, Fructose and Sucrose Contents by HPAEC

Glucose, fructose and sucrose content were assayed by HPAEC (High Performance Anion-Exchange Chromatography). After removing the core, lettuce leaves were frozen in liquid nitrogen, grounded using IKA A11 basic Analytical mill (IKA®, Germany) and then freeze-dried using a Cryotec Cosmos freeze-dryer (Cryotec®, France). Between 15 and 20 mg of dried lettuce were sampled in ultra-pure water to obtain a concentration of 10 mg of DW mL⁻¹. Samples were homogenized and centrifugated 4 minutes at 15 000g, 4°C (Merck 3-16KL, KGaA®, Germany). Supernatant was collected and filtered through PTFE membrane filter (0,2 µm, IC Millex®-LG, Merck KGaA®, Germany). Glucose, fructose, and sucrose contents were assayed using HPAEC (Dionex® ICS-3000, USA) by injecting 5 µL of each sample. Separation was carried out using an IC Dionex CarboPac™ PA1 analytical anion-exchange column (10 µm, 4 x 250 mm, Dionex®, USA). The mobile phase was a solution of H₂O (eluent A) and 250 mM NaOH with 4 mM sodium acetate (eluent B) with A/B ratio of 35:65 (v/v). An isocratic elution mode at a constant flow rate of 0,7 ml min⁻¹ was performed. Sugars were monitored by Pulsed Amperometric Detection (PAD), with a detection peak at 254 nm. Curves of glucose, fructose and sucrose contents were obtained by injecting calibration standards with concentrations of 2,5, 5, 7,5, 10, 12,5, 15 and 20 nmoles. CHROMELEON software v.6.7. was used to analyze the concentration of sugars. The limit of quantification was calculated according to previous study [24]. Total soluble sugars (TSS) content was calculated by summing glucose, fructose, and sucrose contents.

2.5. Photosynthetic and Chlorophyll Fluorescence Parameters

Net photosynthesis (µmol CO₂ m⁻² s⁻¹), stomatal conductance (mmol H₂O m⁻² s⁻¹) and the quantum yield of PSII electron transport, *i.e.* efficiency of the photosystem II (Φ_{PSII} calculated as $(F_m' - F_s)/F_m'$) were measured using a portable photosynthetic analyzer (Head version 1.4.7, LI-COR® 6800, Li-Cor, Inc. Lincoln, NE, USA) equipped with a LI-COR® chamber type 6800-01. F_s is the steady state chlorophyll fluorescence level, F_o' the minimum chlorophyll fluorescence yield in the light-adapted state and F_m' the maximum chlorophyll fluorescence yield in the light-adapted state. Parameters were set at 400 µmoles of photons m⁻² s⁻¹ of light (Red and Blue light, R:B ratio of 9:1) with 420 ppm CO₂. Temperature and VPD were not controlled and therefore remained the same as those in the cultivation chamber (24/18,5°C and 1/0,6 kPa for the light/dark periods). Plants from the rotational cultivation system were placed horizontally to allow for analysis and were placed under lighting conditions similar to those in the cultivation system. All plants were rehydrated every hour to prevent a water deficit bias. Measurements were carried out after 15 days of cultivation on 10 plants, with 2 leaves per plant, so that each leaf was measured every hour. Net photosynthesis and stomatal conductance were measured during an entire lighting period of 16 hours. The efficiency of the photosystem II (Φ_{PSII}) was measured during the first 6 hours of lighting, when it is at its maximum. Measurements were recorded manually every 3 to 5 minutes, with value stability serving as the indicator for recording the data.

2.6. Untargeted Liquid Chromatography Analysis

2.6.1. Sample Extraction

At 31 DAT, 10 ± 0,5mg of freeze-dried lettuce was weighed out in a microcentrifuge tube and extracted with 1,5 ml of MeOH:H₂O (1:1, v:v). Samples were mixed for 10min at 20°C, followed by centrifugation for 5 min at 15000 g and 4°C (Sigma 1-16K centrifuge, Osterode am Harz, Germany). Then, 300 µl of supernatant were collected and dried overnight in a miVac Duo Concentrator (Genevac, Ipswich, UK). The dry extracts were resuspended in 200 µl of ultra-pure water and filtered through a 0,22µm PTFE filter by centrifugation at 10000 g, 4°C for 2min.

2.6.2. Untargeted UPLC-ESI-QTOF-MS^E Profiling

Ultra Performance Liquid Chromatography – Electrospray Ionization – Quadruple Time-of-Flight – Mass Spectrometry^E (UPLC-ESI-QTOF-MS^E) analyses were carried out on an Acquity UPLC I-Class system (Waters®, Milford, MA, USA), hyphenated to a Synapt G2-Si quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters®, Milford, MA, USA). The untargeted profiling was conducted with the chromatographic and mass spectrometry conditions as described in [25]. Each sample was injected three times.

2.6.3. Data Processing and Data Analysis

Principal Components Analysis (PCA), an unsupervised technique, and Orthogonal Partial Least Squares Discriminate Analysis (OPLS-DA), a supervised technique, were used for building the qualitative models in this investigation, following the data processing and curation described by [25]. The analyzed spectral data with a coefficient of variation inferior to 30%, a significant difference with ANOVA ($\alpha=0,05$), a max abundance of at least 750 and a fold change superior to 2 were then studied with PCA using Rstudio [26]. All data were mean centered, and Pareto scaled prior to OPLS-DA. S-plot representation was done to differentiate discriminant molecules between CT and ROT lettuces.

2.7. Postharvest Analyses

At 31 DAT, at harvest, lettuces were minimally processed. Leaves were cut in pieces (approximately 3 x 3cm), washed in chlorinated water (80 ppm) for 3 minutes, rinsed with tap water for 1 minute and then dried. The leaves were packaged in propylene trays covered with oriented polypropylene film using TOP SEAL 160 heat-sealer and stored in standard climatic chambers. Temperature was equal to $5,2 \pm 0,5^{\circ}\text{C}$, measured with Kistock KH 110 sensor. Samples were analyzed after 0, 3, 7 and 10 days of storage. O₂ and CO₂ content were recorded using the Gas CheckMate3 (Dansensor, United Kingdom). The respiration rate was measured with a static system and calculated using the following formula, with Vs representing the volume of the static system in milliliters and FW representing the fresh weight in grams.

$$\text{Respiratory intensity (mmoles h}^{-1} \text{ kg of FW}^{-1}) = (\text{slope of CO}_2 \text{ 100}^{-1}) \times ((V_s - \text{FW}) \times 273) / ((273 + T^{\circ}\text{C}) \times 22,4 \times (\text{FW } 1000^{-1}))^{-1}. \quad (1)$$

Overall visual quality (OVQ) was scored by a three-member trained panel. Quality was evaluated considering wilting severity and overall visual aspect according to a 3 points hedonic scale running from 3=absence of wilting and marketable aspect, to 0 = severe wilting and unmarketable aspect.

2.8. Statistical Analyses

Statistical analyses were carried using RStudio version 4.2.2 [26]. The following packages were used for data analysis and visualization: circlize, ComplexHeatmap, cowplot, dplyr, factoextra, FactoMineR, gdata, ggpattern, ggplot2, ggplotify, ggpubr, ggrepel, grid, gridExtra, multcompView, PMCMRplus, rcompanion, ropls, sf and tidyr. The non-parametric Wilcoxon-Mann-Whitney test was used to determine if the differences observed were significant. The details of the obtained p-values not presented in the figures are provided in the Supplementary Data.

3. Results

3.1. Yields, Efficiencies and Agronomic Parameters

The yields and Water, Light, Energy and Land Surface Use Efficiencies (WUE, LUE, EUE and LSUE, respectively) of CT and ROT lettuces are presented in Figure 2. No significant differences in fresh and dry yield, WUE or LUE were observed. The EUEs of ROT lettuces were equal to 50g of FW kWh⁻¹ and 1,6g of DW kWh⁻¹, which was significantly six and five times higher than those of CT

lettuces, respectively. The LSUEs of ROT lettuces were equal to 975g of FW m⁻² d⁻¹ and 31g of DW m⁻² d⁻¹, which was significantly three times higher than those of CT lettuces, respectively.

The details of fresh weight per plant, dry weight per plant and leaf area of CT and ROT lettuces are presented in Table 1. The mean fresh weights of ROT lettuces were always slightly lower than those of the control, with mean fresh weights 25%, 29%, 16%, 6% and 8% lower than those of the control at 14, 19, 24, 28 and 31 DAT, respectively. However, the fresh weight of ROT lettuces was only significantly lower than those of CT ones at 19 DAT. The same trend can be observed for the dry weight, and the dry weight of ROT lettuces was only significantly lower than that of the control at 14 DAT. Thus, at harvest (31 DAT), no significant differences in fresh weight, dry weight and leaf area were observed between ROT and CT lettuces.

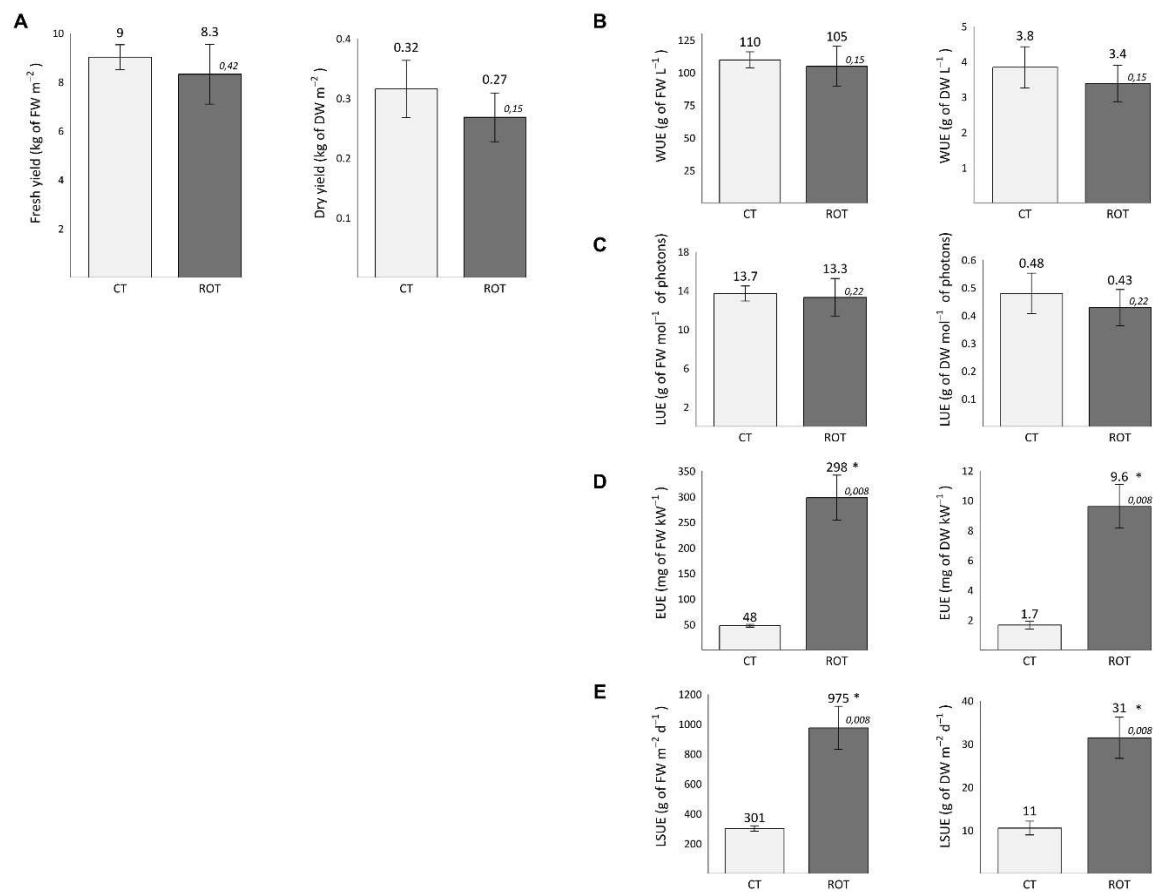


Figure 2. Yields and efficiencies of lettuces grown in control or rotational cultivation systems (CT and ROT lettuces, respectively). (A) Fresh and dry matter yields per square meter. (B) Water Use Efficiency (WUE). (C) Light Use Efficiency (LUE). (D) Energy Use Efficiency (EUE). (E) Land Surface Use Efficiency (LSUE). Efficiencies were calculated according to Orsini et al., 2020. The mean values for CT and ROT lettuces are noted on top of each corresponding bar. Standard deviations are represented as error bars. Significant differences were tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test ($n = 5$, $\alpha = 0,01$). P-values are shown above each bar in italics. Significant differences are indicated by an asterisk..

Table 1. Fresh weight, dry weight, and leaf area of lettuces grown in control and rotational cultivation systems (CT and ROT lettuces, respectively), measured according to days after transplantation (DAT). Data are presented as mean \pm standard deviation (sd). Significant differences were tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test ($n=5$, $\alpha = 0,01$). P-values are shown, and significant differences are indicated by an asterisk.

Fresh weight (g)		Dry weight (g)		Leaf area (cm ²)	
CT	ROT	CT	ROT	CT	ROT

DAT	mean	sd	mean	sd	P-value	mean	sd	mean	sd	P-value	mean	sd	mean	sd	P-value
0	0,4	± 0,07	0,5	± 0,05	0,6	0,02	± 0,005	0,03	± 0,005	0,016	17	± 4	20	± 2	0,151
8	3,3	± 0,29	2,7	± 0,25	0,016	0,23	± 0,02	0,22	± 0,02	0,421	168	± 13	115*	± 8	0,008
14	36	± 4,4	27	± 5,4	0,032	1,9	± 0,12	1,3*	± 0,25	0,008	1051	± 83	795	± 105	0,016
19	112	± 11,9	79*	± 15,8	0,008	4,4	± 0,64	3,4	± 0,57	0,032	2403	± 505	2426	± 499	0,841
24	184	± 18,3	155	± 30,7	0,151	7,5	± 1,07	6	± 1,24	0,0952	3632	± 530	4511	± 1087	0,222
28	262	± 13,3	247	± 8,35	0,095	10,2	± 0,72	8,6	± 0,66	0,0159	7341	± 794	6099	± 884	0,056
31	301	± 16,9	277	± 40,8	0,421	10,5	± 1,6	8,9	± 1,4	0,151	8013	± 1406	6635	± 1351	0,222

3.2. Glucose, Fructose, Sucrose, and Total Soluble Sugars (TSS) Contents

Glucose, fructose, sucrose, and TSS contents are presented in Table 2. At 18 DAT, the glucose, fructose, and TSS contents of ROT lettuces were significantly lower than those of CT lettuces. Moreover, at 31 DAT, the glucose, fructose, and TSS contents of ROT lettuces were lower than those of CT lettuces, but no significant difference was observed.

Table 2. Glucose, fructose, sucrose, and total soluble sugars (TSS) contents of lettuces grown in control and rotational cultivation system (CT and ROT lettuces, respectively), measured at 18 and 31 days after transplantation (DAT). Data are presented as mean ± standard deviation (sd). Significant differences were tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test (n=5 at 18 DAT, n=4 at 31 DAT, α = 0,01). P-values are shown, and significant differences are indicated by an asterisk.

Glucose (mg g ⁻¹ of DW)					Fructose (mg g ⁻¹ of DW)					Sucrose (mg g ⁻¹ of DW)					Total soluble sugars (mg g ⁻¹ of DW)					
CT		ROT		P-value	CT		ROT		P-value	CT		ROT		P-value	CT		ROT		P-value	
DAT	mean	sd	mean		sd	mean	sd	mean		sd	mean	sd	mean		sd	mean	sd	mean		sd
18	108	± 19,8	81*	± 6,4	0,0079	161	± 23,2	101*	± 10,2	0,0079	114	± 21,3	99	± 10,1	0,1508	383	± 23,2	281*	± 15,7	0,0079
31	129	± 14,8	95	± 19,2	0,0635	130	± 16,5	101	± 19,7	0,1111	13	± 2	19	± 8,5	0,2857	273	± 33,2	214	± 32,8	0,0635

3.3. Photosynthetic Parameters

The values of net photosynthesis, stomatal conductance and efficiency of the photosystem II (*ΦPSII*) are presented in Figure 3.

Net photosynthesis and stomatal conductance decreased throughout the lighting period (Figure 3A and B). The net photosynthesis and stomatal conductance of ROT lettuces were significantly lower than those of CT lettuces during the first two hours of lighting with values of 8,6 ± 1,6 and 11,7 ± 0,9 μmol CO₂ m⁻² s⁻¹ for the net photosynthesis and 0,15 ± 0,04 and 0,22 ± 0,04 mmol H₂O m⁻² s⁻¹ for the stomatal conductance, respectively. ROT lettuces net photosynthesis and stomatal conductance of ROT lettuces were also significantly lower than those of CT lettuces at the end of the lighting period, from 10 to 14 hours of lighting. Finally, the net photosynthesis of ROT lettuces was also significantly lower than that of CT lettuces from 14 to 16 hours of lighting. The mean efficiency of the photosystem II (*ΦPSII*) of ROT lettuces during the first 6 hours of lighting was significantly 10% lower than that of the control.

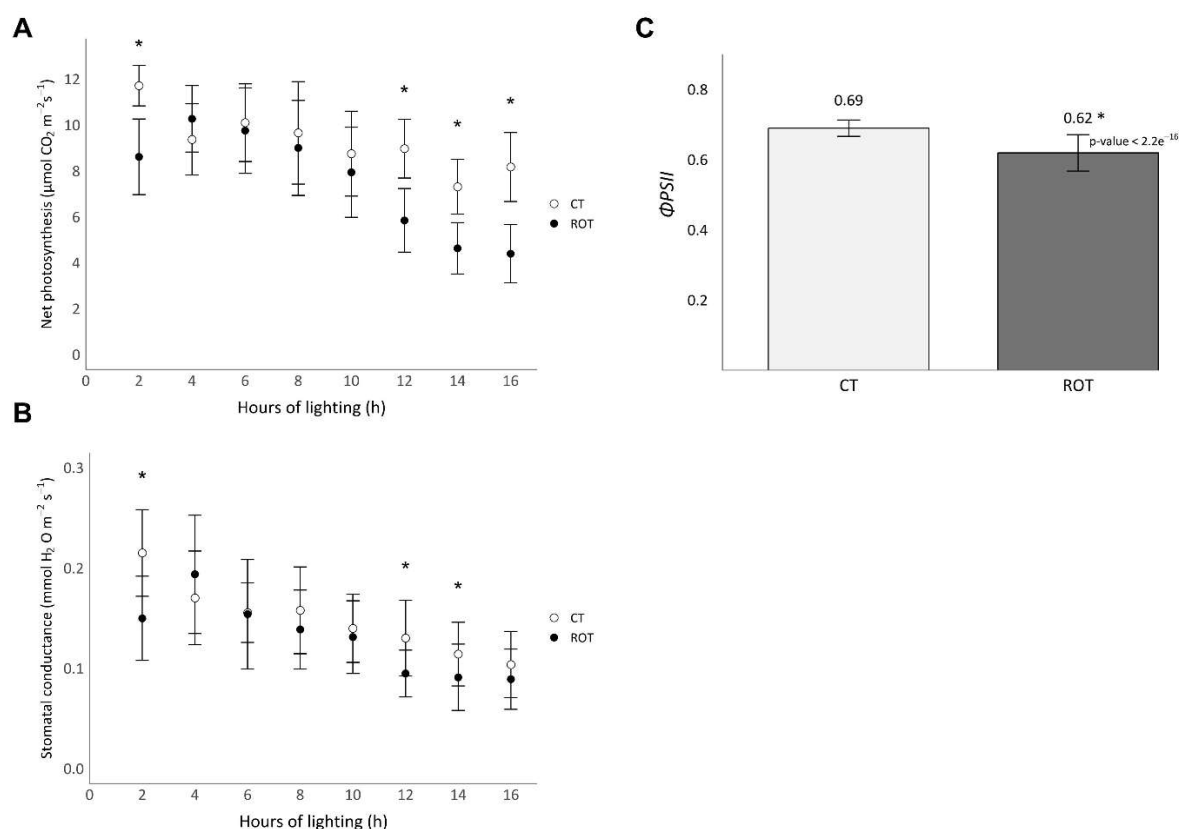


Figure 3. Photosynthetic parameters of lettuces grown in control and rotational cultivation systems (CT and ROT lettuces, respectively). (A) Net photosynthesis, *i.e.*, net carbon assimilation. (B) Stomatal conductance. For (A) and (B), data are expressed according to the 16 daily hours of lighting (h), where 0 represents the beginning of the lighting period and 16 represents the end. Each point represents the mean value of all measurements taken over the previous 2 hours (for each point, $41 \geq n \geq 17$). Standard deviations are presented as error bars. Significant differences were tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test ($\alpha = 0,01$). Significant differences are indicated by an asterisk. (C) Efficiency of photosystem II (Φ_{PSII}), measured during the first 6 hours of lighting. The mean values for CT and ROT lettuces are noted on top of each corresponding bar. Standard deviations are represented as error bars. A significant difference was tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test ($n \geq 50$, $\alpha = 0,01$). P-value is shown, and significant difference is indicated by an asterisk.

3.4. Untargeted Liquid Chromatography Profiling and Main Polyphenols Content at Harvest

PCA analysis, the S-plot derived from the supervised OPLS-DA model, and the peak areas of caftaric acid, chlorogenic acid, and chicoric acid are presented in Figure 4.

In the biplot representation of the PCA (Figure 4A), we observe that samples from ROT lettuces are well separated from those of CT lettuces, as the ellipses representing the groups do not overlap. The first dimension on the x-axis accounts for 64,1% of the total variation across the samples, while the second dimension on the y-axis accounts for 15,2% of the total variation across the samples.

The S-plot from the supervised OPLS-DA model shows a strong involvement of caftaric acid in the differentiation of samples from ROT and CT lettuces (Figure 4B). The caftaric acid peak area was approximately three times lower in samples from ROT lettuces compared to those from CT lettuces (Figure 4C). No significant differences in chlorogenic and chicoric acid peak areas were observed between the two modalities.

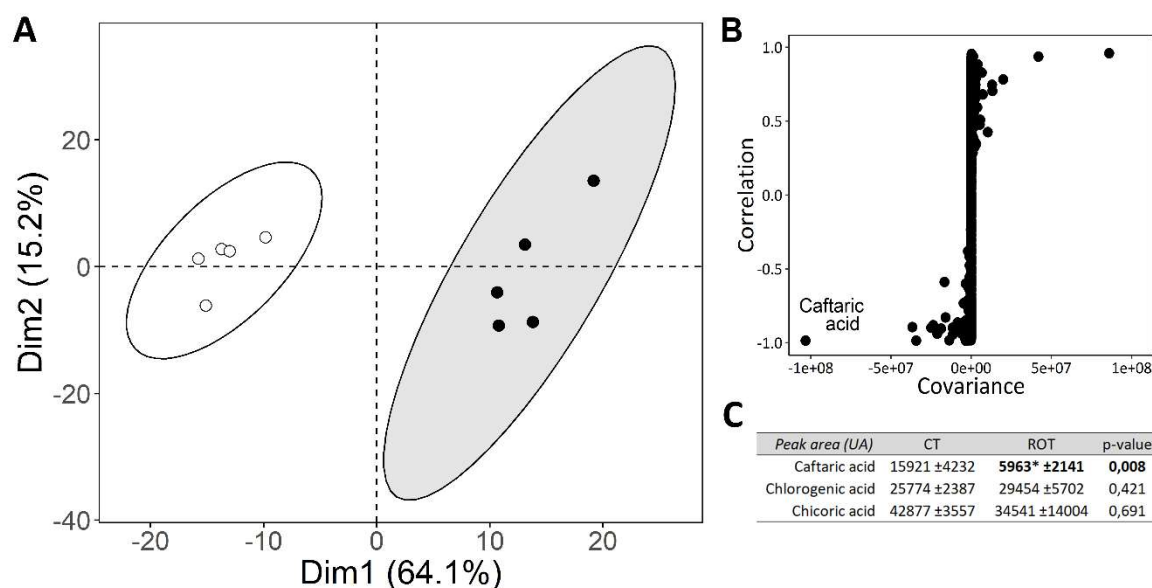


Figure 4. Quality analyses of lettuces grown in control or rotational cultivation systems (CT and ROT lettuces, respectively). (A) Principal Component Analysis (PCA) of the metabolite profiles from untargeted UPLC-ESI-QTOF-MS^E. (B) S-plot from the supervised OPLS-DA model, highlighting caftaric acid as a differentiating metabolite between CT and ROT lettuces. (C) Caftaric, chlorogenic, and chicoric acid contents. Significant differences were tested using the non-parametric Wilcoxon-Mann-Whitney test ($n=5$, $\alpha = 0,01$). P-values are shown, and significant differences are indicated by an asterisk.

3.5. Postharvest Analyses

CO₂ and O₂ packaging content, respiration rates and the overall visual quality (OVQ) of ROT and CT lettuces from harvest until 10 days of storage are presented in figure 5.

The O₂ content inside the packaging of both ROT and CT lettuces decreased throughout the storage period (Figure 5A). Inversely, the CO₂ content inside the packaging of the two modalities increased throughout the storage period (Figure 5B).

Respiration rates of the two modalities decreased from harvest to the third day of storage and then slightly increased until the tenth day of storage (Figure 5C). The respiration rate of ROT lettuces was significantly higher than that of CT lettuces after 3 days of storage, but no significant differences were observed after 7 and 10 days of storage.

Overall visual quality (OVQ) was assessed using a predefined scale, with lettuces graded as non-marketable under a grade of 2 (Figure 5D). No significant differences between ROT and CT lettuces were observed at harvest or throughout the storage period. After 10 days of storage, the grades of ROT and CT lettuces were both above the marketability threshold.

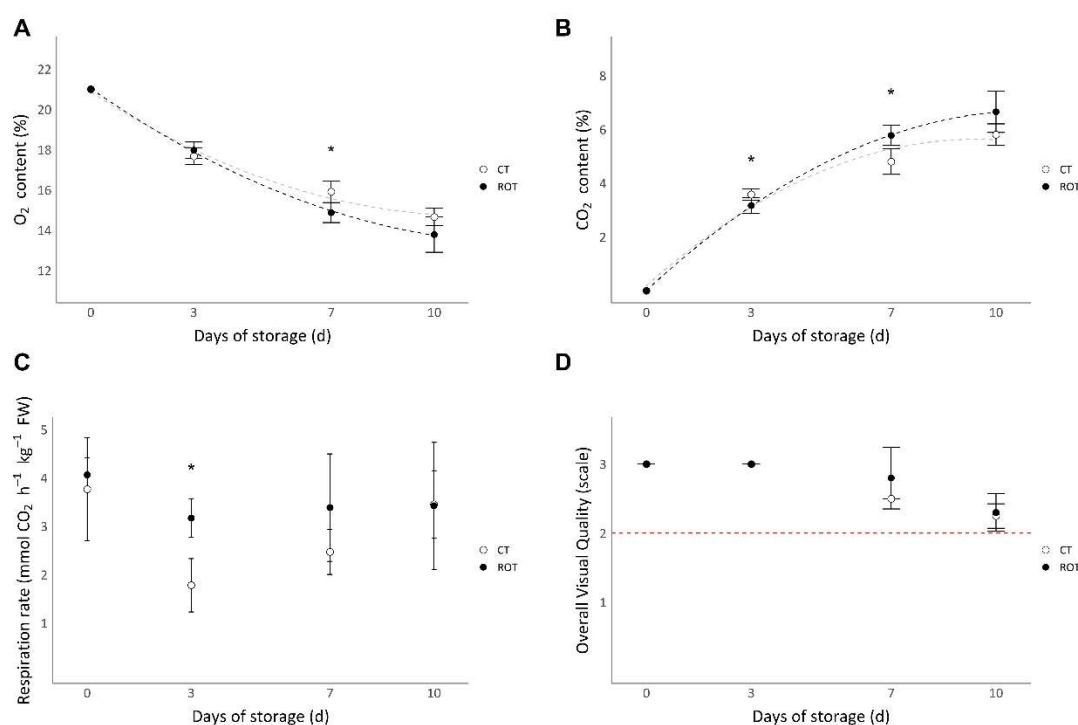


Figure 5. Postharvest parameters of lettuces grown in control and rotational cultivation systems (CT and ROT lettuces, respectively). (A) O₂ content. (B) CO₂ content. For (A) and (B), dashed lines represent the fitting of a second-degree polynomial regression. (C) Respiration rates. (D) Overall visual quality, with the limit of marketability represented by a dashed red bar. Data are represented according to the days of storage. Each point represents the mean value of 10 plants for (A) and (B), and 5 plants for (C) and (D). Standard deviations are represented as error bars. Significant differences were tested using the non-parametric pairwise Wilcoxon-Mann-Whitney test ($\alpha = 0,01$). Significant differences are indicated by an asterisk.

4. Discussion

4.1. The Yields in Control or the Rotational Cultivation Systems Were Similar

In this work, we compared the fresh and dry weights and yields of lettuces grown into two cultivation systems. The first was a control horizontal cultivation system, while the second was a rotational cultivation system, used for indoor production at an industrial scale. Fresh yields obtained in both cultivation systems were similar and equal to 9 and 8,3 kg m⁻², respectively. Those data are consistent with a previous meta-analysis presenting that vertical farming systems can yield up to 6,9 kg of lettuce m⁻² [27]. Moreover, fresh and dry weights obtained at harvest (31 DAT) were consistent with weights of lettuces cultivated in horizontal systems previously described [28].

Similar values in fresh and dry yields were consistent with the fact that there are no significant differences in fresh and dry weights between the two cultivation systems at harvest (31 DAT). However, it must be noted that the mean fresh and dry weights of ROT lettuces were always slightly lower than those of the control, even if significant differences were found at 19 DAT and 14 DAT for fresh weight and dry weight, respectively. Moreover, the mean glucose, fructose, and Total Soluble Sugars contents were lower in lettuces grown in the rotational cultivation system compared to the control ones. Those trends and differences in weights and sugars contents can be explained by net photosynthesis, as the net photosynthesis of lettuces grown in the rotational cultivation system were significantly lower than those of the control in the first two hours of lighting and from ten hours of lighting until the end of the lighting period. Nevertheless, the differences in net photosynthesis were too small to induce significant differences in yields at harvest.

4.2. The Decrease in Net Photosynthesis Observed is a Consequence of the Perturbation of Plants Gravity Perception

To better describe the differences in net photosynthesis between lettuces grown in the rotational cultivation system and the control, we studied the stomatal conductance and the efficiency of the photosystem II. The decrease in photosynthetic activity was synchronous with that in stomatal conductance, which is consistent with the linked between the net photosynthesis and the stomatal conductance previously described [29–31]. Furthermore, the efficiency of the photosystem II ($\Phi PSII$) during the first 6 hours of lighting was 10% lower in ROT lettuces compared to that of CT lettuces. Thus, the observed decrease in net photosynthesis is the consequence of the loss in stomatal conductance and photosystem II efficiency. The rotational cultivation system rotates with a speed of 50 minutes per revolution, which induces a constant perturbation of plant's gravity perception. The perturbation of gravity perception has already been described to decrease net photosynthetic activity of *Triticum aestivum* (wheat) of 25%, and might alter its stomatal conductance [32,33]. Moreover, the activity of the photosystem II of *Triticum aestivum* has already been described to decrease by approximately 10% when the gravity perception of plants are perturbed [34,35]. Based on this literature, it is likely to propose stomatal conductance, photosystem II efficiency and so net photosynthesis decreased because of the perturbation of plant's gravity perception. However, studies on gravity perception are generally focusing on microgravity conditions, due to the high interest for space missions, and that condition of gravity perception might differ from these induced by the rotation cultivation system. Thus, as changes in gravity perception induces drastic and holistic changes in plants [36,37], further research are needed to better define and describe the nature and consequences of the perturbation of gravity perception endured by the plants in the rotational cultivation system.

4.3. Energy and Land Surface Use Efficiencies Were Higher in the Rotational Cultivation System but Still Need to Be Maximized

In this experiment, we compared Water, Light, Energy and Land Surface Use Efficiency (WUE, LUE, EUE and LSUE, respectively) of lettuces grown in a rotational cultivation system (ROT) and a control, in horizontal cultivation system (CT).

The WUE of ROT and CT lettuces were similar and 1,3 and 1,4 times higher than the higher range value presented in for lettuces in vertical farming, respectively [23]. Moreover, the LUE of ROT and CT lettuces were similar. A meta-analysis on Light Use Efficiency of lettuce in vertical farms presented average LUE based on fresh weight of 11,6 g mol⁻¹ and average LUE based on dry weight of 0,55 g mol⁻¹ [38]. In this experiment, we found that LUE based on fresh weight were equal to 13,7 and 13,3 g mol⁻¹ of photons, while those based on dry weight were equal to 0,48 and 0,43 g mol⁻¹ of photons for CT and ROT lettuces, respectively. Thus, the obtained values are consistent with those of literature.

The EUE based on fresh and dry weights of ROT lettuces was approximately six and five times higher than those of the control, respectively. The energy consumption in indoor vertical farming is mainly due to the lighting (range from 60 to 80%) [4,8,10,11]. Two lamps were needed for the rotational cultivation system, while a lamp ceiling was necessary to provide homogenous lighting for the control horizontal cultivation system. Indeed, as lamps are positioned in the center of the rotational cultivation systems, more plants can be illuminated with one lamp. This difference in lamps number might explain most of the difference, however, we were not able to separate lighting-related consumption from that of climate control in both cultivation systems. The EUEs obtained for lettuces grown in the rotational cultivation system were consistent but within the lower range of those reported in recent studies on lettuce. Orsini et al. (2020) reported an EUE range in PFALs from 1 to 140g of FW kWh⁻¹, while Pennisi et al. (2019) reported values ranging from 60 to 100g of FW kWh⁻¹ [23,39]. The EUE obtained in the pilot farm of Futura Gaia Technologies at a pre-industrial scale is close to that obtained in this experiment and is also on the lower end of values reported in the literature, at 48 ± 5 g of FW kWh⁻¹ (internal data, not shown). The energy consumption of lettuces

grown in the rotational cultivation system was equal to $20 \pm 3,0$ kWh kg⁻¹ of FW, which align with previously reported energy consumption ranging from 6,2 to 12,0 kWh kg⁻¹ of FW for lettuces produced in PFALs [40]. It must be noted that, for the control, all data related to energy efficiency are lower and all data related to energy consumption are higher ($119 \pm 6,3$ kWh kg⁻¹ of FW) than the literature, respectively.

The LSUE has been determined to range from 1300 to 3300 g of FW m⁻² d⁻¹ in plant factories with artificial lighting [23]. In this experiment, the land surface use efficiencies in the control and rotation cultivation system were lower than the cited literature as they are equal to 301 and 975 g of FW m⁻² d⁻¹, respectively. Low LSUE can be attributed to the single cultivation layer used in this experiment. In contrast, indoor vertical farming benefits from the ability to multiply cultivation layers. At an industrial scale, up to three rotational cultivation systems can be stacked on top of each other, which lead to an increase in the LSUE. Indeed, the LSUE obtained in the pilot farm of Futura Gaia Technologies is equal to 1252 ± 128 g of FW m⁻² d⁻¹, which makes it more consistent with the literature (internal data, not shown).

In this experiment, WUE and LUE were similar in both systems and consistent with values reported in the literature. This suggests that the resource use efficiency of lettuces in the rotational cultivation system is near optimal. However, the EUE and LSUE values obtained in this experiment and reported from the pilot farm of Futura Gaia Technologies were on the lower end of those reported in the literature. Improvements in space management, system stacking, lighting efficiency, and climate control are needed to significantly increase these values at an industrial scale.

4.4. The Rotational Cultivation System Induced Changes in the Plant's Quality Without Altering the Postharvest Storage Capacity

In the biplot PCA after the LC-MS analysis, we observed a clear separation of the samples coming from lettuces grown in the rotational cultivation system from the control. The conducted analysis focused on polyphenols, and this is consistent with the literature, as it has been reviewed that the growing conditions have an impact on the polyphenols content [21]. Among polyphenols, chicoric acid, biosynthesized from caftaric and chlorogenic acids, is of special interest as it can represent more than 55% of total caffeic acid derivatives [41,42]. No significant differences in chicoric acid were found, but we measured a three times lower content of caftaric acid in ROT lettuces compared to the control. Further research is needed to better characterize the differences in quality of lettuces grown in the rotational and control cultivation system. Moreover, it is of interest to determine if those differences are directly linked with the perturbation in plant's gravity perception.

Higher respiration rate of lettuces grown in the rotational cultivation system compared to the control has been measured only after 3 days of storage, which was consistent with the slight decrease in O₂ content and a slight increase in CO₂ content in ROT lettuces packaging compared to the control during the storage. This might be explained by the rotational cultivation system, as it has been described that perturbation of gravity perception increases the number of mitochondria by at least 100% in *Brassica rapa* and increases the mitochondrial activity and size in *Chlorella* [34,43–45]. However, lettuce from the two modalities were still above the threshold of marketability after 10 days of storage, which shows that the differences in plant's respiration rate did not induce a decrease in plant's overall storage capacity.

5. Conclusions

This study compared lettuce grown in a rotational cultivation system with a horizontal control system, evaluating yields, Water Use Efficiency (WUE), Light Use Efficiency (LUE), Energy Use Efficiency (EUE), Land Surface Use Efficiency (LSUE), and postharvest storage capacity. No significant differences were found in yields, WUE, LUE, fresh or dry weights, or postharvest storage capacity between the two systems. These findings indicate that rotational cultivation is feasible in terms of yields. However, a slight trend toward lower fresh and dry weights in the rotational system was observed, correlating with a decrease in net photosynthesis during certain lighting hours, which

can be explained by reduced stomatal conductance and photosystem II efficiency. Additionally, the rotational system modified the polyphenol profile, and decreased caftaric acid content compared to the control. Thus, further research is needed to better understand its effects on plants physiology and metabolism, particularly regarding the perturbation of gravity perception.

In conclusion, while indoor production using rotational cultivation systems is feasible based on yields, improvements are needed to optimize Energy Use Efficiency and Land Surface Use Efficiency (LSUE), notably through stacking multiple cultivation systems and working on the optimization of the rotation cultivation system itself. Moreover, more specific analyses of energy consumption and energy efficiency are needed to assess the competitiveness and economic feasibility of rotational cultivation systems in industrial-scale PFALs.

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Abbreviations

The following abbreviations are used in this manuscript:

LUE	Light Use Efficiency
WUE	Water Use Efficiency
EUE	Energy Use Efficiency
LSUE	Land Surface Use Efficiency

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