

Article

CO₂ Utilization Strategy for Sustainable Cultivation of Mushrooms and Lettuces

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Abstract: Mushroom cultivation generates a large amount of CO₂ that can be used sustainably. The objective of this study was to use actual cultivation and simulation to find a sustainable cultivation method that uses the CO₂ generated by king oyster mushrooms for the production of romaine lettuces. A closed cultivation system consisting of one mushroom chamber, three lettuce chambers, and one gas-mixing chamber was used. Two cultivation conditions, non-continuous and continuous, were analyzed. The non-continuous system cultivated 15 lettuces and 12 mushroom bottles at a time every 25 and 16 days, respectively. The continuous system cultivated three lettuces and mushroom bottles every five and four days, respectively, so that each chamber contained mushrooms or lettuces at each growth stage. The CO₂ concentrations in the lettuce and mushroom chambers were stably maintained above 1000 μmol·mol⁻¹ and below 2000 μmol·mol⁻¹ in the continuous system. Mathematical models were developed to analyze the CO₂ concentration in each chamber. The shoot dry weight of lettuces grown in the mixed cultivation were 48.0%, 21.9%, 19.7%, and 18.1% at 10, 15, 20, and 25 days after transplanting, respectively, higher than those in the lettuce-only cultivation. Compared to mushroom-only cultivation, mixed cultivation reduced the accumulated CO₂ emissions into the air by 80.6%. Thus, using CO₂ from mushrooms to cultivate lettuce in a continuous cultivation system could reduce CO₂ emissions into the air and enable mixed cultivation of mushrooms and lettuces, achieving sustainable agriculture.

Keywords: canopy photosynthesis; CO₂ balance; CO₂ emission; mushroom respiration; sustainability



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1. Introduction

Increased CO₂ concentration in the atmosphere, which is a driving force of climate change, causes physiological changes in many crops [1]. The impact of elevated CO₂ on plants has been highlighted in connection with climate change, but from a physiological point of view, it is also a factor in increasing crop production [2]. For example, it increases the carboxylation efficiency of plants and affects the photosynthetic rate [3]. In greenhouses, enhanced CO₂ concentrations are actively used to increase crop yields [4,5]. Most CO₂ supply systems in greenhouses involve burning fuel [6]. As a result, many studies have been conducted to find appropriate CO₂ concentration levels for various crops grown in greenhouses [7]. However, some portion of the CO₂ enriched in a greenhouse is released into the atmosphere through ventilation [8]. Therefore, when using CO₂ for crop cultivation, two considerations are needed to enable sustainable cultivation: no use of fossil fuels and no release of CO₂ into the atmosphere.

Because mushrooms are aerobic fungi, their respiration during cultivation produces a large amount of CO₂ that is generally emitted into the atmosphere as a greenhouse gas [9]. High CO₂ concentration suppress the growth of king oyster mushrooms, causing physiological disorder [10], but they positively affect the photosynthesis of plants [11]. Therefore, a previous attempt used the CO₂ generated during shiitake mushroom cultivation for lettuce cultivation [12]. However, the amounts of CO₂ emitted by the shiitake mushrooms

and consumed by the lettuces were not quantified in that report. To accurately analyze the change in CO₂ concentration in the cultivation system, mixed cultivation of mushrooms and lettuces must be continued for a long period. Although Jung et al. (2014) measured the change in CO₂ concentration according to growth stage and ratio of romaine lettuces to king oyster mushrooms [13], they did not precisely analyze the CO₂ behavior. A recent study grew basil plants, shiitake mushrooms, and black soldier fly larvae at the same time, and observed the change in CO₂ concentration in each growth chamber [14], but they did not present cultivation methods to precisely control CO₂ emission and consumption. To analyze this kind of research, it is important to use crops that can quantitatively represent the photosynthesis or respiration depending on environmental factors. King oyster mushrooms and romaine lettuces are suitable crops because their respiration and photosynthesis are expressed in models, respectively [15,16].

For sustainable cultivation, the amounts of CO₂ emitted by mushrooms and consumed by lettuces should be equal over time. To efficiently manage a total cultivation system for both mushrooms and lettuces, it is necessary to quantify the CO₂ emitted and consumed and to establish models of CO₂ behavior in the cultivation system. One possibility is to use continuous (conveyer) cultivation in which plants at several growth stages are simultaneously grown in the cultivation system [17]. That method produces smaller fluctuations in CO₂ concentration than does the non-continuous method, which begins growing all the plants at one time. However, continuous cultivation systems are technically difficult to manage because various environmental variables are associated with one another, and their interactions cannot be tracked with ease [18]. For an adequate investigation of such complicated systems, simulation methods are essential [19]. There have been several attempts to grow multiple organisms simultaneously [12–14], but no precise predictions have been attempted by using simulation. Thus, the objective of this study was to use actual cultivation and simulation to find a sustainable way to use the CO₂ produced by respiration of king oyster mushrooms to grow romaine lettuces.

2. Materials and Methods

2.1. Plant Materials and Cultivation Conditions

Romaine lettuces (*Lactuca sativa* L. cv. Asia Heuk Romaine) and king oyster mushrooms (*Pleurotus eryngii* (DC.) Quél) were used for the experiment. Yamazaki's nutrient solutions with an electrical conductivity of 1.2 dS·m^{−1} were applied to the lettuces. The inside temperature and photosynthetic photon flux density (PPFD) of the lettuce growth chamber were maintained at 24 °C and 200 μmol·m^{−2}·s^{−1}, respectively, with an 8:1:1 ratio of RBW light-emitting diodes (LEDs). The photoperiods in the lettuce growth chamber were set to 16 h (day) and 8 h (night). The mushroom chamber was kept dark using a black cloth and the inside temperature and humidity were maintained at 18 °C and 95%, respectively.

2.2. Mushroom and Lettuce Mixed Cultivation Systems

For simulation and evaluation of the mushroom-only and mixed (lettuce and mushroom) cultivations, a cultivation system consisting of one mushroom chamber, one mixing chamber, and three lettuce chambers (Figure 1) was constructed. The size of the lettuce and mushroom chambers was 400 L (1.0 × 0.8 × 0.5 m) and that of the mixing chamber was 125 L (0.5 × 0.5 × 0.5 m). Each chamber was made of acrylic plates and sealed. The ventilation number of each chamber was 0.039 h^{−1}, which was negligible in calculating CO₂ concentration. Lettuces at 0, 5, 10, 15, and 20 days after transplanting (DAT) and mushrooms at 3, 7, 11, and 15 days after scratching (DAS) were used for the mixed cultivation system.

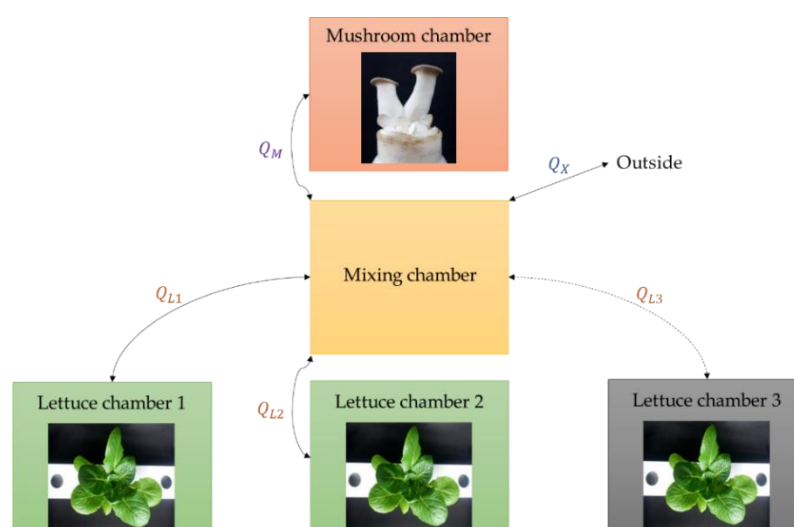


Figure 1. Conceptual diagram of a cultivation system consisting of one mushroom chamber, one CO₂ mixing chamber, and three lettuce chambers. The arrows and symbols indicate the air flow and air flow rates, respectively.

Air was circulated between the chambers at a flow rate of 62 L·min^{−1} using diaphragm pumps (Boxer 7004, Uno International Ltd., London, UK). CO₂ generated in the mushroom chamber was circulated to the lettuce chambers via the mixing chamber. The mixing chamber was designed to exchange air between the chambers and the external air in case the CO₂ concentration in the total system become too high or low. The CO₂ concentration in each chamber was measured every two minutes using an infrared CO₂ sensor (LI-820, LI-COR, Lincoln, NE, USA) and was recorded using a data logger (CR1000, Campbell Scientific, Logan, UT, USA). The pumps between lettuce and mixing chambers, between mushroom and mixing chambers, and between mixing chamber and the external air were operated when the CO₂ concentration fell below 1000 μmol·mol^{−1} in the lettuce chamber or rose above 2000 μmol·mol^{−1} in the mushroom chamber or the mixing chamber. AC/DC controllers (SDM-CD16AC, Campbell Scientific Inc., Logan, UT, USA) were used to control the diaphragm pumps.

2.3. CO₂ Behavior Models for the Cultivation Systems

The photosynthetic rates in the lettuce chambers (Equation (1)) were calculated using models of lettuce photosynthesis over time experimentally developed by Jung et al. (2016) [16]. The photosynthetic rate model was determined by the regression analysis based on a rectangular hyperbola equation. The model contains variables to operate at different CO₂ levels and growth stages.

$$P_n = \frac{a \times b \times C_{Ln}}{a + b \times C_{Ln}} + c \quad (1)$$

where a is the photochemical efficiency (μmol·mol^{−1}), b is the carboxylation conductance (s^{−1}), and c is the dark respiration (μmol·m^{−2}·s^{−1}). Values for a , b , and c were obtained by a regression analysis with photosynthetic rates at each growth stage. Therefore, a , b , and c are expressed as exponential functions according to the growth stage, and they are finally organized in the form of Equation (2). The estimated coefficients showed an accuracy of $R^2 = 0.99$ and $RMSE = 1.2 \mu\text{mol}\cdot\text{s}^{-1}$ [16].

$$P_n = \frac{21.812 \times e^{-0.057 \times t} \times e^{-0.050 \times t} \times C_{Ln}}{57.4 \times e^{-0.057 \times t} + 0.380 \times e^{-0.050 \times t} \times C_{Ln}} - 18.608 \times e^{-0.056 \times t} \quad (2)$$

The CO₂ emission rate in the mushroom chamber (Equation (3)) was calculated using exponential functions developed by Chanter and Thornley (1978) [20]. The respiration rate model was determined by the regression analysis based on an exponential equation. The model contains variables to operate at different growth stages of mushrooms. The coefficients of the models were experimentally determined by using the mushroom respiration measured in this system.

$$R = M \times W + Y \times \frac{dW}{dt} \quad (3)$$

where R is the respiration rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), M is the maintenance coefficient (s^{-1}), W is the dry weight (g), and Y is the CO₂ production coefficient ($\text{g} \cdot \text{g}^{-1}$). Since the king oyster mushrooms in this study were harvested before reaching the stationary phase, dry weight is expressed as an exponential function to the growth stage, and it is organized in the form of Equation (4). The estimated coefficients showed an accuracy of $R^2 = 0.64$ and $\text{RMSE} = 21.6 \mu\text{mol} \cdot \text{s}^{-1}$ [15].

$$R = 0.016 \times e^{0.549 \times t} - 48.352 \quad (4)$$

The following equations were used to express the CO₂ behavior in the cultivation systems:

$$V \times \frac{dC_{Ln}}{dt} = (C_X - C_{Ln}) \times Q_{Ln} - k \times LN_n \times \sum P_n \quad (5)$$

$$V \times \frac{dC_M}{dt} = (C_X - C_M) \times Q_M + k \times MN \times \sum R \quad (6)$$

$$V_X \times \frac{dC_X}{dt} = (C_M - C_X) \times Q_M + (C_O - C_X) \times Q_X + \sum ((C_{Ln} - C_X) \times Q_{Ln}) \quad (7)$$

The parameters and coefficients used in the models are defined in Table 1. The initial CO₂ concentrations in the lettuce, mushroom, and mixing chambers were 1000, 500, and 1000 $\mu\text{mol} \cdot \text{mol}^{-1}$, respectively. The external CO₂ concentration was assumed to be 500 $\mu\text{mol} \cdot \text{mol}^{-1}$, because the CO₂ concentration in the building where the experimental system was installed was about 500 $\mu\text{mol} \cdot \text{mol}^{-1}$.

Table 1. Units and values of parameters and coefficients used in the CO₂ behavior models.

Parameter	Description	Unit	Value
V	Volume of the lettuce or mushroom chamber	L	400
V_X	Volume of the mixing chamber	L	125
Q_{Ln}	Flow rate between the lettuce chamber n and mixing chamber	$\text{L} \cdot \text{min}^{-1}$	62
Q_M	Flow rate between the mushroom and mixing chambers	$\text{L} \cdot \text{min}^{-1}$	62
Q_X	Flow rate between the mixing chamber and external air	$\text{L} \cdot \text{min}^{-1}$	62
k	Unit conversion factor at 20 °C (CO ₂ $\mu\text{mol} \cdot \text{mol}^{-1}$ to CO ₂ $\text{g} \cdot \text{L}^{-1}$)	$\text{g} \cdot \text{L}^{-1} / (\mu\text{mol} \cdot \text{mol}^{-1})$	1.83×10^{-6}
LN_n	Number of lettuces in the chamber n at a specific growth stage	ea	15
MN	Number of mushroom bottles in the chamber at a specific growth stage	ea	12
C_{Ln}	CO ₂ concentration in the lettuce chamber n	$\mu\text{mol} \cdot \text{mol}^{-1}$	
C_M	CO ₂ concentration in the mushroom chamber	$\mu\text{mol} \cdot \text{mol}^{-1}$	
C_X	CO ₂ concentration in the mixing chamber	$\mu\text{mol} \cdot \text{mol}^{-1}$	
C_O	CO ₂ concentration in the external air	$\mu\text{mol} \cdot \text{mol}^{-1}$	500
P_n	Photosynthetic rate of a lettuce at a specific growth stage in the chamber n	$\mu\text{mol} \cdot \text{s}^{-1}$	
R	Respiration rate of a mushroom at specific growth stage in the chamber	$\mu\text{mol} \cdot \text{s}^{-1}$	
t	Days after transplanting (DAT) for lettuce and days after scratching (DAS) for mushroom	day	
n	Lettuce chamber number		1, 2, 3

2.4. Simulation Conditions for the Non-Continuous and Continuous Cultivation

Non-continuous and continuous cultivation systems were analyzed and compared. In the non-continuous system, 15 lettuces and 12 mushroom bottles were placed at a time in the lettuce and mushroom chambers every 25 and 16 days, respectively (Figure 2A). In the continuous system, three mushroom bottles and lettuces were placed in the lettuce and mushroom chambers every 5 and 4 days, respectively, so each chamber contained mushrooms at 4, 8, and 12 DAS or lettuces at 5, 10, 15, and 20 DAT (Figure 2B). In both cases, the mushroom chamber contained 12 bottles of mushrooms, and each lettuce chamber contained 15 lettuces. In the simulation, the air flow rate and photoperiod of each chamber were set to match the conditions in the actual chambers. The photoperiods in the lettuce chambers were set as shown in Table 2 for continuous cultivation to ensure that photosynthesis occurred in two lettuce chambers at all times. Simulations were conducted in MATLAB (Mathworks, Natick, MA, USA) to estimate the CO₂ concentration in each chamber for 50 days.

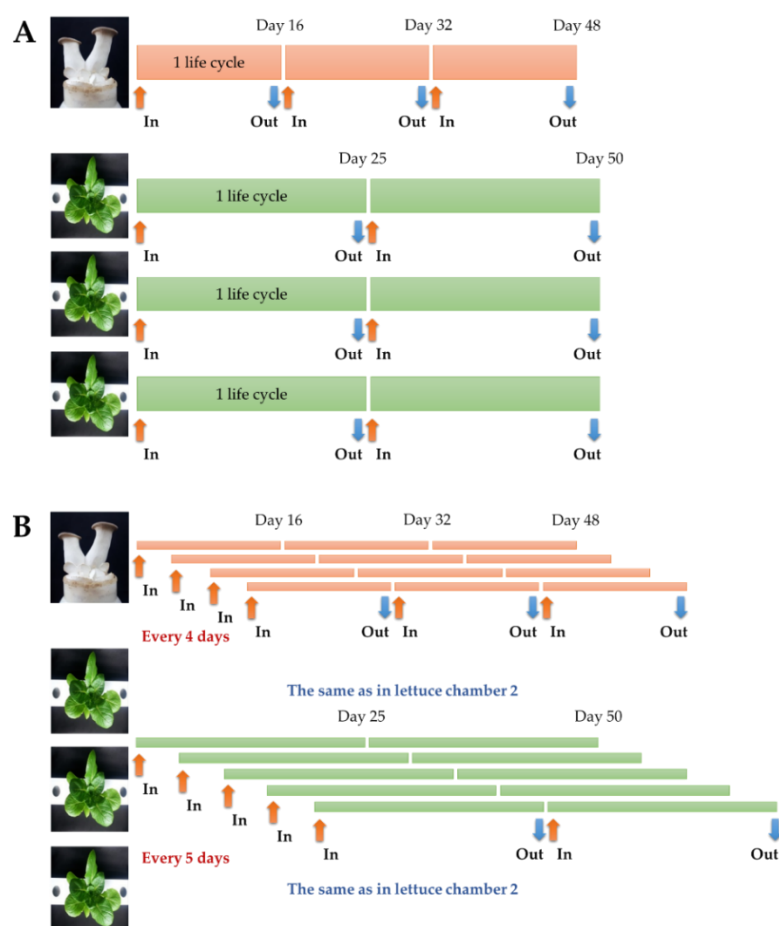


Figure 2. Schematic diagram of the non-continuous cultivation (A) and continuous cultivation (B) systems. The total number of lettuces and mushrooms in each chamber at any time is 15 and 12, respectively.

Table 2. Photoperiods in the three lettuce chambers for the continuous cultivation system.

Period	Chamber 1	Chamber 2	Chamber 3
00:00–08:00	Dark	Light	Light
08:00–16:00	Light	Dark	Light
16:00–24:00	Light	Light	Dark

2.5. Evaluation of Continuous Cultivation

Two experiments were conducted for 5 days and repeated three times. The number of mushrooms and lettuces in each chamber was set to be the same as the previous simulation conditions. First, the shoot fresh and dry weight and leaf area of the lettuces grown in the mixed, continuous cultivation system (Figure 1) and those grown in the lettuce-only chamber, which were where maintained at $400 \mu\text{mol}\cdot\text{mol}^{-1}$, were compared at each growth stage. The collected samples were dried in an oven at 70°C for three days and shoot dry weights were measured. The environmental conditions in the chambers were the same except for CO_2 concentration. Second, the accumulated CO_2 emissions from the mixing chamber in the mixed, continuous cultivation system (Figure 1) and those from the mushroom-only chamber maintained at a CO_2 concentration below $2000 \mu\text{mol}\cdot\text{mol}^{-1}$ were compared.

2.6. Statistical Analysis

All measured and simulated data were evaluated using the SPSS statistical package (IBM, New York, NY, USA). Data were analyzed with Duncan's new multiple range test (DMRT) after an analysis of variance (ANOVA) at the significance level of 0.05.

3. Results and Discussion

3.1. Simulated CO_2 Behavior in Non-continuous and Continuous Cultivation Conditions

The CO_2 concentrations simulated in the mushroom and lettuce chambers for 50 days showed a difference between non-continuous and continuous cultivation (Figure 3). With non-continuous cultivation, the CO_2 concentration in the lettuce chambers remained above $1000 \mu\text{mol}\cdot\text{mol}^{-1}$ for 400 h. After that, a rapid imbalance depleted the CO_2 concentration in the lettuce chambers. The CO_2 concentration seemed to be restored at about 600 h, but it was depleted again after 800 h. This was due to the large amount of CO_2 consumption by the photosynthesis of the lettuces compared to that produced by the respiration of the mushrooms. With continuous cultivation, on the other hand, the CO_2 concentration in the lettuce chambers remained constant at $1000 \mu\text{mol}\cdot\text{mol}^{-1}$ throughout the period. Thus, the CO_2 concentration with continuous cultivation was well balanced. This result was similar to previously published predictions with wheat and radish [17], implying that a mixed continuous cultivation system is an advantageous method that offers sustainable control of CO_2 behavior.

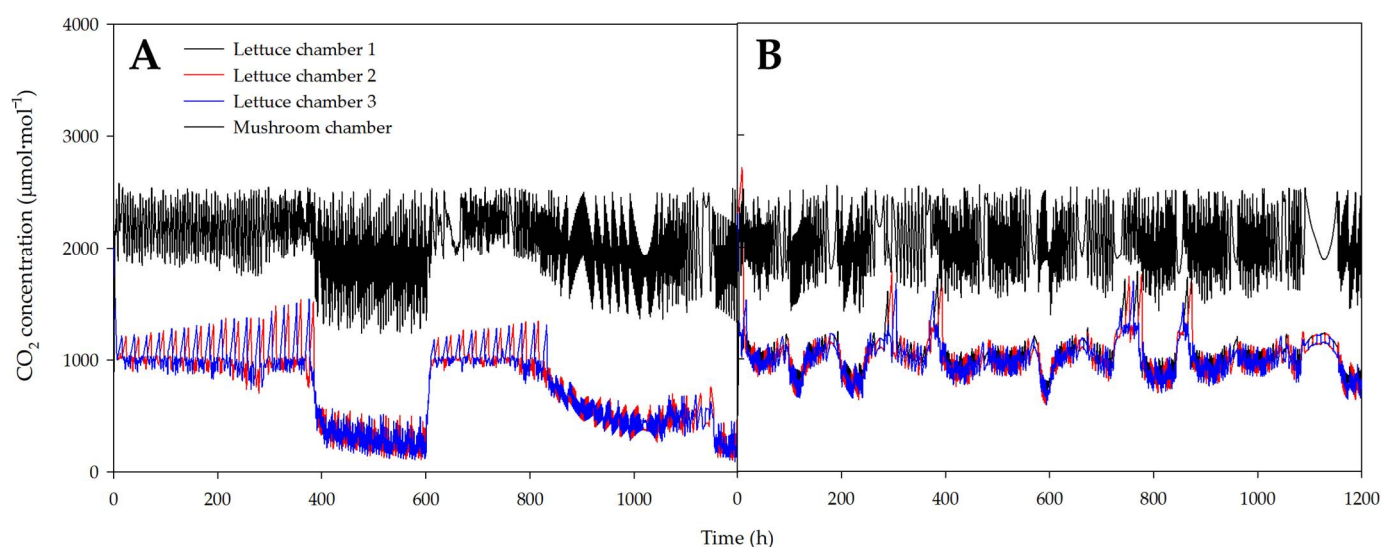


Figure 3. Simulated CO_2 concentrations in the three lettuce chambers and the mushroom chamber with non-continuous cultivation (A) and continuous cultivation (B) for 50 days.

3.2. Evaluation of Continuous Cultivation of Lettuce and Mushroom

The measured and simulated CO₂ concentrations in the lettuce and mushroom chambers were compared (Figure 4). The CO₂ concentrations in the lettuce chambers were controlled to the set point of 1000 $\mu\text{mol}\cdot\text{mol}^{-1}$ or higher (Figure 4A–C). In addition, the CO₂ concentration in the mushroom chamber stayed below the set point (Figure 4D). The CO₂ concentration in the mixing chamber fluctuated more frequently than the simulation had predicted, but no dramatic breakaway was observed (Figure 4E). At night in each lettuce chamber, the measured CO₂ concentration was lower than in the simulation. Because the respiration rate of the actual lettuces was lower than that of the simulated lettuces, the CO₂ leakage from the chambers used in the experiments was negligible. However, it is believed that the difference between the measured and simulated CO₂ concentrations occurred because the leakage from the pipes connecting the chambers was not reflected.

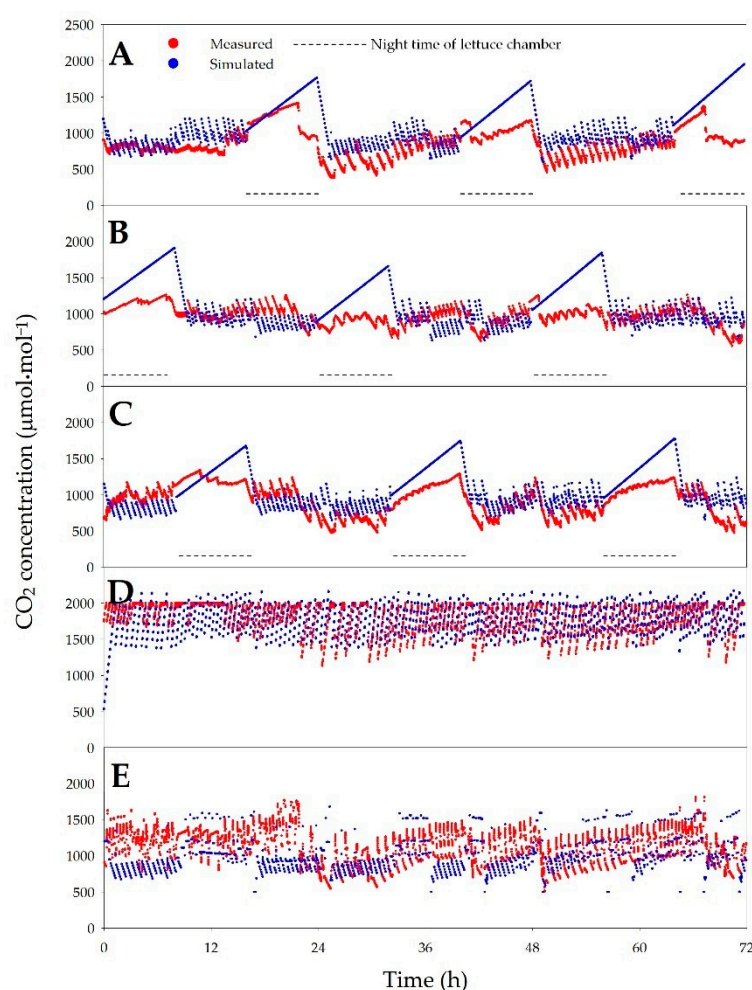


Figure 4. Measured (red dots) and simulated (blue dots) CO₂ concentrations in lettuce chambers 1 (A), 2 (B), and 3 (C); the mushroom chamber (D); and the mixing chamber (E) with continuous cultivation.

The results confirmed that gas exchange took place through the pre-operation of pumps during the dark period in the lettuce chambers, and the CO₂ concentration in the mushroom chamber was well controlled below the set point of 2000 $\mu\text{mol}\cdot\text{mol}^{-1}$. When the CO₂ concentration in mushroom cultivation facilities is below 2400 $\mu\text{mol}\cdot\text{mol}^{-1}$, the marketable values of king oyster mushrooms is maximized [21]. Therefore, when applying the continuous cultivation method, CO₂ concentration should be maintained at appropriate levels.

The shoot fresh and dry weight and leaf area of lettuces grown in the mixed, continuous cultivation system were significantly higher than those grown using lettuce-only cultivation except for leaf area at 25 DAT (Figure 5). The shoot dry weight of lettuces grown in the mixed cultivation were 48.0%, 21.9%, 19.7%, and 18.1% higher at 10, 15, 20, and 25 DAT, respectively, than those in the lettuce-only cultivation. Compared with the shoot fresh weight of lettuce grown at a plant factory for three weeks [22], the lettuce in this experiment showed no physiological disorder. That result is consistent with existing research showing that lettuce grown at a PPFD of $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and enhanced CO_2 concentration increased in fresh weight [23]. In addition, the supply of CO_2 promotes the growth of lettuce, which shortens the harvest time. Therefore, these results show that lettuce can be grown without fossil fuels using CO_2 generated from mushroom respiration.

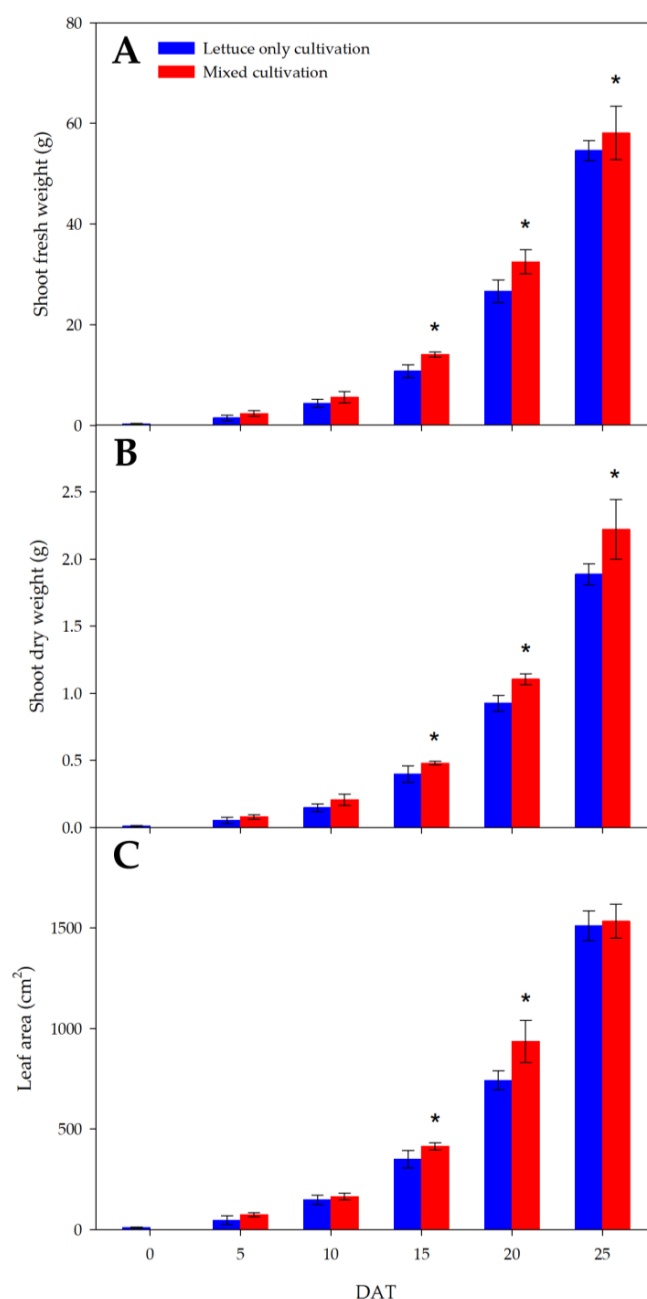


Figure 5. Shoot fresh weight (A), shoot dry weight (B), and leaf area (C) of lettuces grown in lettuce-only cultivation (blue) and mixed, continuous cultivation (red). Bars represent mean \pm standard deviation ($n = 15$). An asterisk (*) indicates a statistically significant difference (ANOVA/Duncan) ($p < 0.05$).

3.3. Estimation of Accumulated CO₂ Emission into the Air

The accumulated CO₂ emissions from the mixing chamber into the air were estimated to be 35 g for five days, but the actual measured value was 48 g (Figure 6). Because CO₂ was emitted only when the pumps were operating, the accumulated CO₂ emissions over time appeared step-like. With mushroom-only cultivation, the accumulated CO₂ emissions were 180 g during the same period. Therefore, the mixed cultivation system reduced CO₂ emissions into the air by 80.6%. Thus, using CO₂ from mushroom respiration for plant photosynthesis effectively reduces the CO₂ released into the atmosphere.

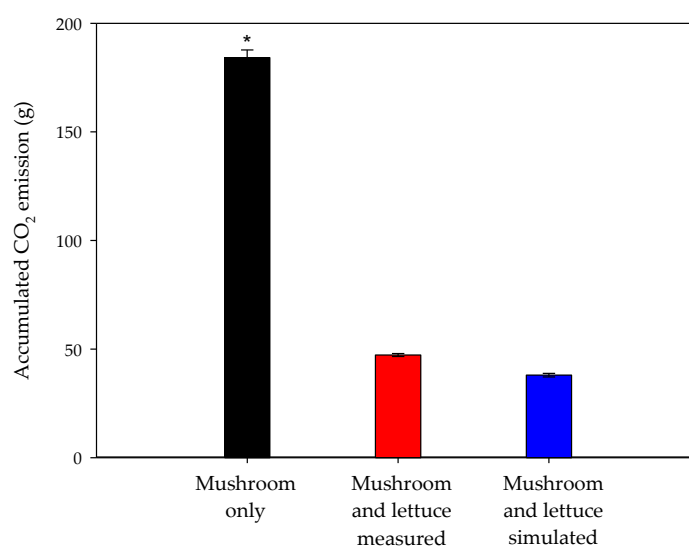


Figure 6. Accumulated CO₂ emissions into the atmosphere over 5 days. Bars represent mean \pm standard deviation ($n = 3$). An asterisk (*) indicates a statistically significant difference (ANOVA/Duncan) ($p < 0.05$).

Anthropogenic CO₂ emissions continue to increase, resulting in rising global temperatures and sea levels, and acidification of the oceans [24]. According to the climate change scenario, there are some predictions that if anthropogenic CO₂ emissions are totally eliminated, global temperatures will not rise [25]. The agricultural sector accounts for about 15% of the total greenhouse gas emissions from human activities [26]. These emissions are mostly due to methane and nitrogen oxides from livestock farming. For sustainable agriculture, it is recommended to minimize soil disturbance, carry out crop rotation, and manage fertilizer and nutrients [27]. However, among the many sub-sectors of agriculture, common carbon reduction strategies often do not work. One example is mushroom cultivation on commercial farms that emit CO₂ in high concentrations, and the other is some greenhouse cultivation, which uses high CO₂ concentrations to improve crop productivity. Recently, sophisticated respiration and photosynthesis models have been used to represent CO₂ emission and consumption [15,16]. Therefore, attempts to reduce CO₂ emissions from agricultural activities by using modeling and simulation are required to continue.

4. Conclusions

CO₂ concentrations in the lettuce and mushroom chambers were estimated by the simulation of mixed, continuous cultivation. CO₂ concentrations in the lettuce and mushroom chambers were experimentally verified and shown to be controlled within allowable ranges. The shoot dry weight of lettuces grown in mixed cultivation were 48.0%, 21.9%, 19.7%, and 18.1% higher at 10, 15, 20, and 25 days after transplanting, respectively, than those in lettuce-only cultivation. With the mixed, continuous cultivation system, CO₂ emissions into the atmosphere could be reduced by 80.6% compared with the total CO₂ emitted by mushrooms through respiration. Because CO₂ is one of the factors responsible for global warming, minimizing CO₂ emissions caused by mushroom cultivation will be meaningful.

The results in this study enable a reduction in CO₂ emissions as well as improvements in plant production, achieving sustainable agriculture and mitigating climate change.

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