REGACE – Crop Responsive Greenhouse Agrivoltaics System with CO2 Enrichment for Higher Yields

Project Deliverable Report

DELIVERABLE 2.2 – CO2 ENRICHMENT SYSTEMS

WORK PACKAGE NUMBER: 2

WORK PACKAGE TITLE: IMPLEMENTATION

TYPE: REPORT

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REGACE Action Information	
Action full title	Crop Responsive Greenhouse Agrivoltaics System with CO2 Enrichment for Higher Yields
Action acronym	REGACE
Grant agreement number	101096056
Project coordinator	Prof. Ibrahim Yehia
Project start date and duration	1 February 2023, 36 months
Project website	https://regaceproject.com/

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Document Information

Deliverable Information	
Work package number	**2**
Work package title	**Implementation**
Deliverable number	**2.2**
Deliverable title	**CO2 Enrichment Systems**
Description	**Report on planning, installation and optimization of the CO2 enrichment systems in the greenhouses of the different locations.**
Lead beneficiary	**UBER**
Lead Author(s)	**Thorsten Rocksch**
Contributor(s)	**Ibrahim Yehia – Alzahrawy, Norbert Keutgen – BOKU, Chrysoula Papaioannou – UTH, Max Liebrich - BW**
Revision number	**1**
Revision Date	**2026/02/02**
Status (Final (F), Draft (D), Revised Draft (RV))	**F - Final**
Dissemination level (Public (PU), Restricted to other program participants (PP), Restricted to group specified by consortium (RE), Sensitive - limited under the conditions of the Grant Agreement (SEN))	**PU**

Document History			
Revision	**Date**	**Modification**	**Author**
0.1	2025/12/05	Outline	Thorsten Rocksch
0.2	2026/01/09	Initial draft	Thorsten Rocksch Jens Neumann Uwe Schmidt
0.3	2026/01/16	Inputs from technology leaders	Ibrahim Yehia Norbert Keutgen Chryssoula Papaioannou
1.0	2026/02/02	Final version for submission	Thorsten Rocksch

Approvals				
	Name	**Organisation**	**Date**	**Signature (initials)**
Coordinator	Prof. Ibrahim Yehia	Alzahrawy Society	2026/02/02	IY
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List of Abbreviations

BW – Watzkendorf organic farm (Germany)
BOKU – University of Natural Resources and Life Sciences, Vienna
CCS – Carbon Capture and Storage
CCU / SSU – Carbon Capture and Utilization (CO_2 capture and utilization)
CH_4 – Methane
CO – Carbon monoxide
CO_2 – Carbon dioxide
CO_2BAG – CO_2BAG® Finland CO_2 Products Oy (commercial CO_2 bag product)
CO_2Bags / CO2Bags – CO_2 releasing bags used for enrichment
Ct – Total carbon content (carbon of transfer mulch sample)
CTRL – Control treatment
DAT – Days after transplantation
H_2O – Water
L – Litre
LCA – Life Cycle Assessment
m^2 – Square metre
m^3 – Cubic metre
N_2O – Nitrous oxide
NH_3 – Ammonia
NH_4HCO_3 – Ammonium hydrogen carbonate
NOx – Nitrogen oxide
OCAP – CO_2 ring pipeline system near Rotterdam (OCAP CO_2 pipeline)
ppm – Parts per million
PV – Photovoltaic / Photovoltaics
PV Tracking – Photovoltaic tracking (solar tracking PV modules)
PV Tracking + CO_2 – Solar tracking PV modules with simultaneous CO_2 enrichment
RH – Relative humidity
Rubisco – Ribulose 1,5 bisphosphate carboxylase/oxygenase
RuBP – Ribulose 1,5 bisphosphate
SE / S.E. – Standard error
SO_2 – Sulfur dioxide
SOx – Sulfur oxides
TBAB – Tetrabutylammonium bromide (in TBAB + CO_2 semi clathrate hydrate system, referenced)
UBER – Humboldt University site in Berlin
UTH – University of Thessaly (Volos, Greece)
$W\ m^{-2}$ – Watt per square metre
WP – Work Package
µmol mol^{-1} – Micromole per mole (gas concentration unit)

Executive Summary

This report compares and evaluates a range of CO_2 enrichment strategies tested in greenhouse systems across several European and Mediterranean research and demonstration sites, including UBER and BW (Germany), BOKU (Austria), UTH (Greece) and Alzahrawy Society (Israel). Both technical and biological approaches were investigated to determine their effectiveness, controllability, and practical relevance under different climatic and operational conditions.

At Humboldt University (UBER), technical CO_2 enrichment using bottled gas and high-precision sensors enabled accurate and stable maintenance of target concentrations between 400 and 800 ppm in phytoboxes and a research greenhouse. This allowed reliable monitoring of plant responses and calculation of net photosynthesis, demonstrating a highly controllable, research-grade system.

At Watzkendorf organic farm (BW), CO_2 release was achieved through the application of transfer mulch composed of grasses and rye straw. Laboratory incubation confirmed substantial conversion of carbon to CO_2 over time, indicating potential as a low-input biological source, although emission rates were variable and difficult to control.

At BOKU Vienna, two biological methods were tested. CO_2 bags produced only modest increases in CO_2 concentration but led to yield improvements in some crops, potentially influenced by both CO_2 and ammonia released from the bags. Mushroom cultivation successfully elevated CO_2 levels by approximately 30–35%, but results clearly showed that increased CO_2 only improved photosynthesis and yield when sufficient light intensity was available.

At UTH in Volos, a fully automated technical CO_2 system using pressurized cylinders consistently raised CO_2 levels by about 100 ppm during enrichment periods while maintaining stable temperature and humidity. However, yield differences among treatments remained statistically insignificant, suggesting that other environmental factors limited productivity.

At Alzahrawy in Israel, technical CO_2 enrichment was effective mainly during early morning hours. High temperatures and the resulting need for ventilation quickly reduced CO_2 concentrations, restricting the overall impact of enrichment.

Overall, the findings demonstrate that while technical CO_2 systems provide the most reliable and controllable enrichment, biological approaches offer low-cost alternatives with limited predictability. Across all sites, light availability, temperature, and ventilation emerged as key limiting factors, and elevated CO_2 alone was insufficient to overcome suboptimal growing conditions.

Chapter 1 – Introduction

History of CO_2 enrichment in greenhouses

The carbon content of the atmosphere has long been known to be a limiting factor in photosynthesis. The positive effects of CO_2 on plant growth were described as early as 200 years ago (Panwar, Kaushik, & Kothari, 2011). From 1900 to the 1930s, extensive research was conducted in numerous European countries and the US on the effects of CO_2 on plant development (Mortensen, 1987). CO_2 enrichment was specifically used to promote plant growth. As early as the 1920s and 1930s, CO_2 began to be deliberately introduced into greenhouses. However, the process was not established until the 1960s, primarily in the Netherlands (Wittwer & Robb, 1964). In the 1970s, CO_2 enrichment was also introduced in greenhouses in Scandinavian countries, initially mainly for lettuce production. In 1986, CO_2 enrichment systems were already being used in 75% of greenhouses in Norway (Moe & Mortensen, 1986). In the Netherlands, CO_2 enrichment systems became widespread in greenhouses. In 1995, it was reported that 80% of Dutch greenhouse operations used CO_2 enrichment systems, 50% of which did not combine them with heating (Esmeijer, 1999). Over time, CO_2 enrichment systems were introduced in numerous countries. Currently, they are commonly found in the Netherlands, Germany, Spain, France, the United Kingdom, the United States, and South Korea (Villagran et al., 2025; Wang, Lv, Wang, & Shi, 2022; Y. Zhang et al., 2023). CO_2 enrichment is often an integral part of climate control and crop management in greenhouses.

Use of various CO_2 sources

The CO_2 requirement for greenhouse crops is considerable. Studies from the Netherlands estimate that 5-6 million tons of CO_2 are required for a greenhouse area of 10,000 hectares (Vermeulen, 2014). Various sources are available for the use of CO_2.

Fossil fuel combustion

For a long time, CO_2 enrichment systems were primarily used in combination with the combustion of fossil fuels. The combustion of natural gas is a very suitable measure in this context, as it releases few gases that are harmful to plants. Combustion gases from oil burners, on the other hand, are not suitable for CO_2 enrichment. They contain too many harmful gases such as sulfur dioxide (SO_2). The combustion of 1m³ of natural gas produces approximately 1.8 kg of CO_2. To prevent the formation of harmful gases due to incomplete combustion of natural gas, more air is added to it than would theoretically be required for complete combustion. This is referred to as excess air. The excess air is indicated by an "air factor" (ll). An ll of 1.1 means an excess air ratio of 10%. The CO_2 content of the combustion gases depends on this air factor and is around 9% for a small flame (ll = 1.3) and up to 10.5% for a large flame (ll = 1.1). If there is no excess air (ll = 1) but complete combustion takes place, this is referred to as stoichiometric combustion (Esmeijer, 1999). The gas discharged from the boiler usually contains too much heat and sometimes gases that are harmful to plants, such as NOx, SO_2, and CO. Efficient cooling and cleaning processes are therefore essential (Li, Ding, Li, & Miao, 2018). When using central natural gas-fired boilers, the exhaust gases with a CO_2 content of approx. 10% are extracted from the chimney by additional metering fans, mixed with fresh air, and blown into the greenhouses. In general, all gas burners used in horticultural technology are suitable for CO_2 enrichment. The dosing value is between 30 m³ and 150 m³ of natural gas per hectare per hour (Esmeijer, 1999). However, it should be noted that a significant proportion of the CO_2 introduced into the greenhouse is not utilized by plant photosynthesis but is vented again, especially when global radiation values are high. PVC pipe distribution systems are generally used to ensure even distribution.

However, one disadvantage of the combined use of central heating systems for firing and CO_2 enrichment is the uneven demand for CO_2 and thermal energy. Less heating energy is required in the summer months, and during the day, the highest CO_2 demand is during daylight hours, while at night, the heating demand is higher. During the summer months, less heating energy is required during the day than at night, and CO_2 demand during the day depends on the intensity of radiation. One possible solution would be to use CO_2 storage facilities that collect enough CO_2 at night when the heating is on and release it during the day when plants have a high CO_2 demand (Takeya et al., 2017). In warm areas, little heating power is required, so CO_2 production from fossil fuel emissions is not cost-effective under these conditions (Li et al., 2018). Storing CO_2 requires large storage volumes. Even when the gas is compressed, this is much more complex than storing heat in water heat storage tanks. That is why in many greenhouse facilities with CO_2 enrichment from the combustion of natural gas, the burners are operated during the day, the CO_2 is fed into the greenhouse, and the heat energy generated in the process is stored in an insulated water tank for heating purposes during the night.

Directly fired CO_2 burners (CO_2 cannons) are attached to the gable end of the greenhouses and run on natural gas or propane gas. A very high degree of efficiency can be achieved here because there are no heat losses due to piping between the boiler and the greenhouse. These systems are primarily used for CO_2 fertilization and less for heating. As a rule, outside air is fed to the burner for the combustion process.

Use of technical CO_2

CO_2 supply via tanks and cylinders

Pure, liquefied CO_2 is often used for fertilization and can be used for enrichment in greenhouses. In general, liquefied CO_2 is fed into the greenhouses from a storage tank or cylinders by means of a controlled valve. The main advantage of using technical CO_2 is the ability to precisely control the dosage to maintain the CO_2 concentration in the greenhouse at an optimal level (Kuroyanagi, Yasuba, Higashide, Iwasaki, & Takaichi, 2014). Even though the use of technical CO_2 is cost-intensive, with suitable control strategies this form of CO_2 enrichment can be more profitable than enrichment from combustion gases (Chalabi, Biro, Bailey, Aikman, & Cockshull, 2002). However, the use of technical CO_2 requires gas storage and pressure control equipment, which takes up space in greenhouses and requires regular maintenance. When stored in tank facilities in greenhouse complexes, which are generally used for areas larger than 3000 m², this equipment must be inspected by technical inspection associations.

CO_2 supply in combination with industrial plants

In connection with the fulfilment of climate protection requirements, there is global interest in reducing or avoiding CO_2 emissions. Large industrial plants generate significant amounts of CO_2 during combustion processes. Utilizing the CO_2 produced by industrial processes in greenhouses can be an important step toward implementing the CCS or SSU strategy (CO_2 capture and utilization). Near Rotterdam (Netherlands), a CO_2 ring pipeline (OCAP) has been in place since 2005, supplying greenhouses in the Westland region with CO_2 from industrial waste gases (Ros, Read, Uilenreef, & Limbeek, 2014). As of 2018, approximately 1,900 hectares of greenhouse space are supplied with CO_2, and 500,000 tons of CO_2 are used annually for CO_2 enrichment in greenhouses. This corresponds to a saving of 140 million m^3 of natural gas (Rezaei, Burg, Pfister, Hellweg, & Roshandel, 2024).

Use of CO2 sources from renewable energies

Biomass

The use of biomass is another environmentally friendly way of enriching greenhouses with CO_2. Biomass combustion produces more CO_2 than natural gas, but it also contains higher levels of harmful gases, particularly NO_X and SO_X compounds. However, the use of membrane filters and special exhaust air treatment can significantly reduce the proportion of gases that are harmful to plants (Dion, Lefsrud, & Orsat, 2011).

Biogas

The combination of biogas and greenhouse facilities represents another opportunity for CO_2 enrichment, particularly in rural areas. In biogas plants, organic matter is typically broken down under anaerobic conditions to produce biogas, which can then be burned to generate heat and CO_2 for agricultural operations and greenhouses. This combined approach offers further advantages; for example, an LCA study in which biogas is used to substitute natural gas and for CO_2 enrichment shows that greenhouse gas emissions such as CO_2, CH_4, and N_2O can be reduced by more than 80% on an annual average. In addition, digestate from biogas plants can be used as fertilizer for plant production (S. Zhang, Bi, & Clift, 2015). However, the same challenges arise as with biomass combustion in terms of harmful gas content. Therefore, air filtration is also required before CO_2 utilization of the exhaust gases in biogas combustion.

Use of ambient air as a natural source of CO_2

Another source of CO_2 for plant production in greenhouses is the ambient air that enters the interior of the greenhouse through open ventilation or forced ventilation. With closed ventilation, CO_2 concentrations in greenhouses are sometimes only 100–150 ppm, compared to outdoor concentrations of around 400 ppm (Kozai, Kubota, Takagaki, & Maruo, 2015). These low CO_2 concentrations significantly impair the photosynthetic performance of plants in the greenhouse. For this reason, growers sometimes open the ventilation before the target ventilation temperature is reached to increase the CO_2 concentration in the greenhouse through natural ventilation. Alternatively, forced ventilation can be used, where the air exchange can be adjusted relatively precisely using speed-controlled fans (Montero, Stanghellini, & Castilla, 2009). When using ambient air via ventilation systems, CO_2 distribution in greenhouses is usually very good. However, the maximum achievable CO_2 concentration is that of the ambient air.

Practical application

When using CO_2 in greenhouses, other factors must be considered in addition to choosing a suitable CO_2 source. The interaction of the plant with CO_2 and the interaction with other growth factors or cultivation conditions is crucial for the successful use of CO_2 enrichment.

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Photosynthesis is a relatively inefficient process, as only around 8-10% of the energy from sunlight is converted into chemical energy in the form of reduced sugars. Added to this are further losses due to autotrophic respiration and limitations in other growth factors (water, plant nutrients, etc.), which reduce the actual efficiency of the conversion to around 2-4% (Long 2006; Zhu 2010). One approach to improving plant growth is to increase the rate of photosynthesis through CO_2 enrichment and the associated potential acceleration of plant growth. Under optimal growth conditions, the photosynthesis rate can be increased by more than 50% at elevated CO_2 concentrations compared to plants under normal CO_2 concentrations (Kirschbaum, 2011). Depending on location and season, the CO_2 concentration in the atmosphere is approximately 400-420 ppm (Hoheisel, 2023). Under average or suboptimal growing conditions, the photosynthesis rate increases by more than 40% at elevated CO_2 concentrations. Over the whole day, this value is reduced to an average of 30%. However, an increase in the photosynthesis rate only increases the growth rate of the plant by about 10% (Kirschbaum, 2011).

One of the limitations lies in photosynthesis itself, so a distinction must be made between Rubisco-limited photosynthesis rates and ribulose 1,5-bisphosphate (RuBP) regeneration-limited photosynthesis rates. The relative photosynthetic response to increased CO_2 concentrations gradually decreases with rising temperatures. The Rubisco limitation of photosynthesis at low intercellular CO_2 concentrations changes to RuBP regeneration limitation at elevated CO_2 concentrations. At low radiation levels, photosynthesis is also generally limited by RuBP regeneration (Kirschbaum, 2011). According to Zuh et al. (2010), it can be assumed that most plants are adapted to pre-industrial CO_2 concentrations and are therefore less Rubisco-limited than RuBP regeneration-limited. RuBP regeneration limitation at elevated CO_2 concentrations would therefore be one of the decisive growth limitations under most growth conditions. Most measurements of photosynthesis rates under experimental conditions are carried out under high light irradiation (light saturation point) and at higher temperatures, where the test plants are presumably Rubisco-limited under these experimental conditions and photosynthesis is maximized by CO_2 enrichment. At lower temperatures, radiation levels, or in plants that partially shade themselves due to their habit, the photosynthesis rate will be significantly lower. It can therefore be assumed that the experimentally measured slopes of the photosynthesis rate are significantly higher than the actual photosynthesis rates under normal conditions (Drake, 1997; Kirschbaum, 2011).

Another limitation arises from sink-source interaction within the plant. Additional carbon assimilated through CO_2 enrichment can only be converted into improved growth if the plant has the appropriate consumers through new leaf development, root growth, or seed formation. If a plant is sink-limited, increased carbon assimilation through CO_2 enrichment can only be maintained to a limited extent and is restricted by corresponding feedback processes; this process is referred to as downward acclimatization. Assimilated carbon can only be converted into active growth if other nutrients relevant for plant growth are available in sufficient concentrations. When considering plant growth, it therefore makes sense to assume that the plant nutrient or growth factor that is least available to the plant is also the limiting factor for plant growth (law of the minimum).

Another influencing factor is the growth phase of the respective plants. In early stages of development, plants initially grow exponentially until they have increased in size, partially shade themselves, and exponential growth transitions into linear growth. A slight increase in the growth rate due to CO_2 enrichment during the exponential development phase can lead to higher biomass. However, this depends on the initial growth rate of the plant (fast vs. slow growing) and the nutrient supply (Kirschbaum, 2011).

Chapter 2 – CO$_2$-Enrichment at UBER

At the Humboldt-University (UBER) in Berlin, two different CO$_2$ enrichment systems were utilised using technical CO$_2$ via bottles. Phytoboxes (fig.1) were used for exact preliminary tests. Here, the CO$_2$ enrichment was checked and the technology for precise CO$_2$ enrichment was optimized. High-quality CO$_2$ sensors with an accuracy of 5 ppm are used. The flow rate can be manually regulated and read during operation (fig.2). This makes it possible not only to keep the CO$_2$ concentrations in the Phytoboxes at a constant value, but also to calculate the net photosynthesis rate of the plants.

Figure 1 Three identical phytoboxes with precise control

Control of CO$_2$, light, temperature, and humidity

Figure 2 CO2 control system

Left - screenshot of control software; Centre - CO$_2$ cylinders supplying phytoboxes, Right - adjustable flow valve.

In the experiments with the phytoboxes, the specified target values for CO$_2$ concentrations were accurately maintained; the results of the experiments are presented in detail in D3.6.

Fig. 3 shows a screenshot of the control panel for the phytoboxes: The CO$_2$ target values of 400, 600, and 800 ppm are precisely maintained during the day. At night, the CO$_2$ content rises above the target value of 400 or 600 ppm due to plant respiration; only at 800 ppm is a CO_2 supply required.

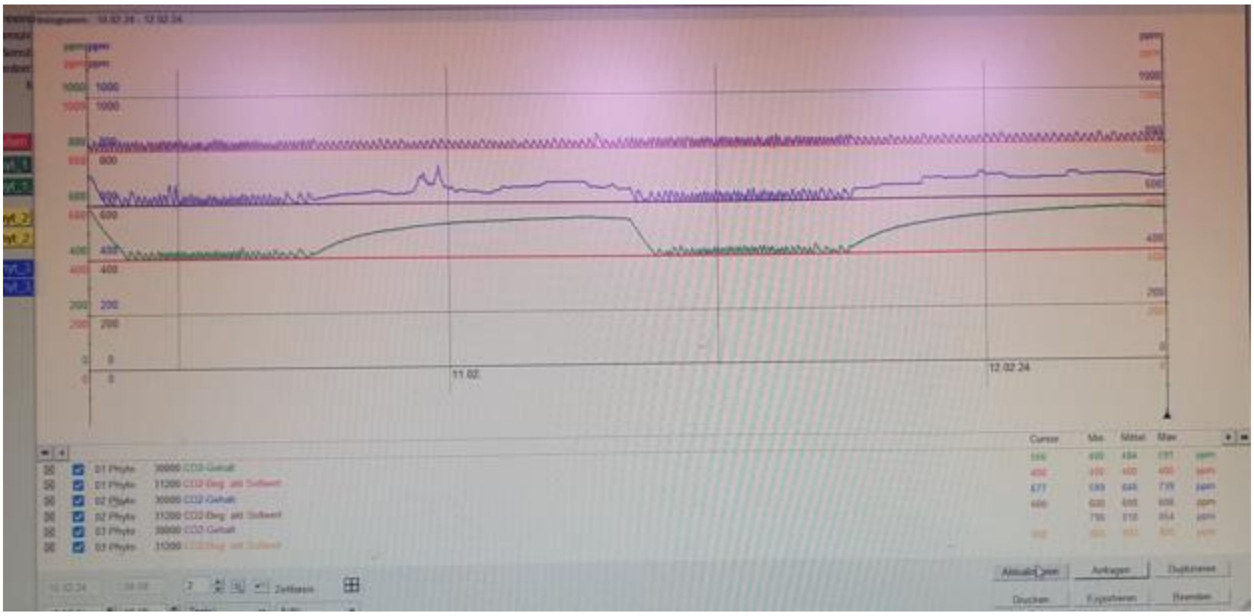

Figure 3 Target and actual values of the CO2 concentrations

In the research greenhouse where the PV modules are installed, CO_2 is also supplied via cylinders connected as a bundle (Fig. 4). The CO_2 supply is an integral part of the greenhouse's climate control system. CO_2 concentrations can be measured at five points in the greenhouse at a height of 1.5 m, as well as vertically using a sensor array at a height of 1–6 m. In addition, two further CO_2 sensors have been installed on the PV modules. A phytomonitoring system records the CO_2 concentrations in the leaf environment (Fig. 5), which allows for the calculation of net photosynthetic performance (see D3.3).

Figure 4 Greenhouse views UBER

Left: exterior of research greenhouse, Centre: interior view with energy screen, Right: CO_2 cylinders bundled.

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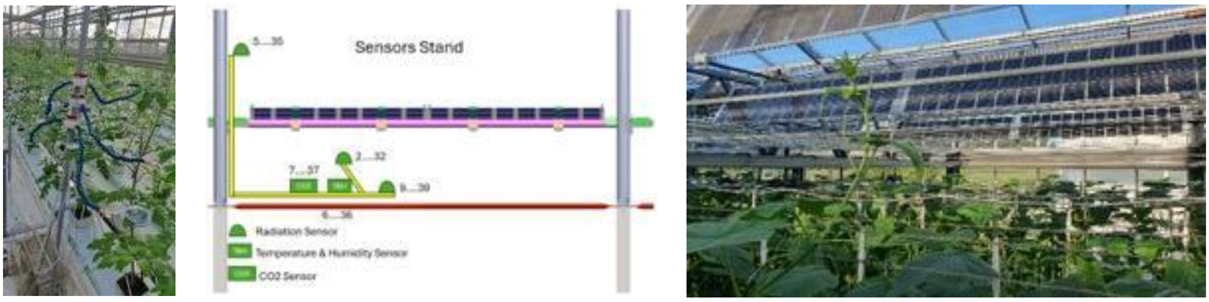

Figure 5 Phytomonitoring system

Left: Phytomonitoring system with leaf cuvettes; Centre and Right: Placement of the CO_2 sensors on the PV module.

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Chapter 3 – CO_2-Enrichment at BW

At the Watzkendorf organic farm (BW), CO_2 enrichment is achieved by spreading transfer mulch. The transfer mulch consists of a mixture of various grasses and rye straw. Before cultivation in spring, approximately 5 kg/m² of transfer mulch is placed on the soil of the greenhouses and not tilled into the soil (Fig. 6). The effect of CO_2 release was recorded in 2024 and 2025 via CO_2 measurements in the greenhouse.

Figure 6 Greenhouse views BW

Left - Placement of the PV modules in BW, Right - Transfer mulch layer in cucumber cultivation.

The potential CO_2 release of the transfer mulch was quantified in 2025 using a system for measuring potential soil respiration (Fig.7). Here, material samples are incubated over a longer period of time. This also allows the dynamics of CO_2 release to be recorded.

Figure 7 Unit for measuring CO2-emission; transfer mulch sample for CO2 absorption

The carbon content of the transfer mulch was determined prior to the incubation experiment. The C_t content was 44.9%. With a sample weight of 2 g, this corresponds to 898 mg of carbon. After a period of 35 days, CO_2 releases of between 561 and 683 mg were detected in a total of 20 samples (Fig.8). Detailed results can be found in D3.6

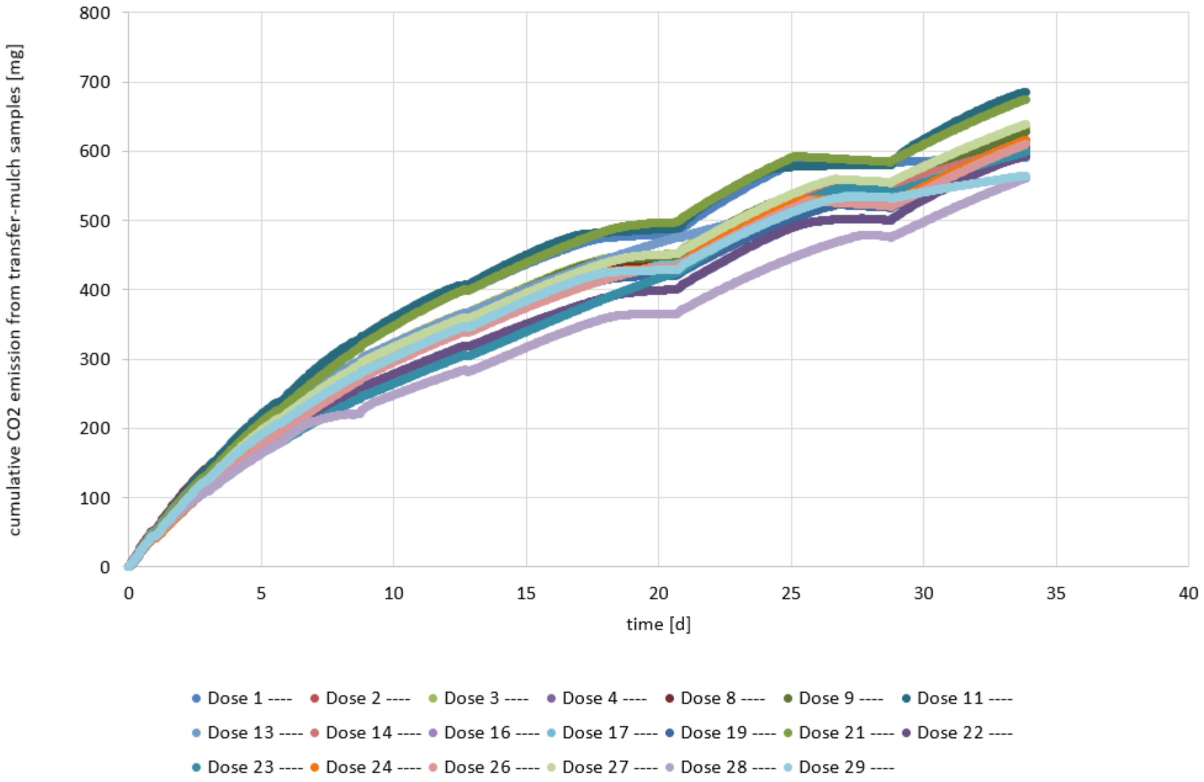

Figure 8 Cumulative CO2 emissions

From 20 transfer mulch samples (each sample weight 2 g).

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Chapter 4 – CO_2-Enrichment at BOKU

At BOKU, two options for elevating the atmospheric CO_2 levels in the greenhouse cabins were tested, first by the application of CO_2Bags (CO2BAG®Finland CO2Products Oy), and second by the production of CO_2 by mushrooms cultivation in a nearby unit, from where the CO_2 was continuously supplied to the plants. CO_2 concentrations were measured with the EGM-5 CO_2 Gas Analyser from PP Systems (USA) within the canopy in the morning between 9.00 and 10.00 o'clock, at midday and in the afternoon between 14.00 and 15.00 o'clock.

CO_2-enrichment by CO_2-Bags

For the experiments, the CO_2-Bags were placed between or above the plants – depending on the size of the plants – such as recommended by the producer of the bags. On average, eight CO_2Bags were used per 5 m². Temperature regulation of the greenhouse cabins was not interrupted, resulting in the opening of the cabin windows when temperature rose above the threshold value of 25 °C. This should have reduced elevated CO_2 levels further. The CO_2-Bags were exchanged every three weeks.

Table 1 Mean CO2 concentration in cabins with CO2-Bags

Significances are based on paired T-Tests & Bonferroni correction. Different letters in a row indicate significant differences at the 5% probability level.

Period considered	C3 (shading) [ppm]	C4 (shading & CO_2) [ppm]	C5 (control) [ppm]	Crop
17/05/-15/06/2023	433 ± 25 a	440 ± 21 a	452 ± 56 a	*Ocimum basilicum* L. 'Hohes Grünes Basilikum'
23/03/-15/08/2023	464 ± 48 a	464 ± 41 a	477 ± 47 a	*Capsicum annuum*
16/05/-17/07/2023	456 ± 33 a	458 ± 38 a	471 ± 47 a	*Lactuca sativa* cv. Teide
06/02/-21/03/2024	446 ± 15 b	467 ± 20 a	440 ± 21 b	*Ocimum basilicum* L. 'Hohes Grünes Basilikum'
06/03/-11/05/2024	442 ± 26 b	459 ± 38 a	438 ± 30 b	*Raphanus sativus* L. cv. Riesen von Aspern
06/03/-29/05/2024	439 ± 26 b	455 ± 39 a	435 ± 30 b	*Cucumis sativus* L. cv. Snackgurke Hopeline F1
06/03/-31/08/2024	424 ± 24 a	434 ± 48 a	410 ± 18 b	*Capsicum annuum* L. cv. California Wonder
22/03/-16/05/2024	439 ± 26 b	456 ± 38 a	436 ± 29 b	*Raphanus sativus* L. cv. Riesen von Aspern

The effect of CO_2-enrichment by CO_2Bags was comparatively small, being insignificant in 2023 (Table 1). Although significant differences occurred in the 2024 experiments, the CO_2 concentration in the cabin with CO_2Bags was only about 20 µmol mol^{-1} higher than in the other cabins (Table 1). In summary, in 4 out of 8 experimental approaches was the CO_2 concentration significantly increased and in 5 out of 8 experiments, a significant increase in yield was achieved by using CO_2Bags compared to the shading variant without CO_2Bags: in basil (2x), cucumber (1x), lettuce (1x) and radish (1x). Noteworthy, in one of the mentioned

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basil experiments and in the case of lettuce, differences in the CO_2 levels were not observed despite differences in yield, which might be regarded as an argument in favour of the hypothesis that the effect on yield was not necessarily limited to the CO_2 released by the CO_2Bags. With respect to quality aspects, the addition of CO_2Bags tended to increase leaf carbohydrate content. In addition, there was also a tendency towards higher nitrate levels in some of the crops.

Noteworthy, the substance in the CO_2Bags is ammonium hydrogen carbonate (NH_4HCO_3), which reacts to form NH_3, CO_2 and H_2O. The NH_3 content in the air close to the crops was measured during one week with a Picarro G2103 NH_3 Gas Analyser (Santa Clara, California, USA) indicating a tenfold concentration increase in the cabin with the CO_2Bags (Fig. 9). This may indicate that either additional CO_2 or NH_3 or both could have affected the growth response and quality of crops.

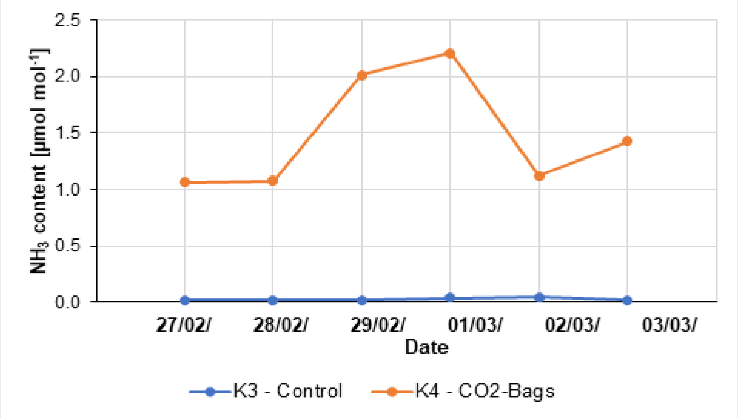

Figure 9 NH3 concentration

Measured within the plant stand in a shaded cabin with and without CO_2Bags.

It is suggested that the positive effect of CO_2Bags on yield could be attributed not only to slightly elevated CO_2 levels but also to ammonium as a foliar nitrogen fertiliser or a combination of both. Further studies are needed to investigate this phenomenon. However, based on the yield increases observed at BOKU, it is doubtful at this stage, whether the cost/benefit ratio of CO_2Bags – the latter costs *c*. 15-20 € per bag – justifies their use in commercial greenhouse cultivation.

CO_2 enrichment by mushroom cultivation

In a preliminary experiment in two 400 L climate cabins (Tritec GmbH, Hanover, Germany), one cultivation box each of button and oyster mushrooms (company Pilzmännchen, Malschwitz, Germany) were investigated for their CO_2 emission potential in order to scale up this experiment to the volume of a cabin in the BOKU greenhouse of 78 m^3.

The experiment revealed increasing CO_2 levels within the first three weeks, then a distinct drop and stable emissions resulting in cabin CO_2 concentrations between 1000 and 1500 ppm (Fig. 10). For Regace, a mushroom cultivation unit of *c*. 5 m^3 volume close to cabin 4 (shading & CO_2) was established, where 50 substrate bags inoculated with oyster mushrooms supplied by Hut & Stiel (Vienna, Austria) were cultivated.

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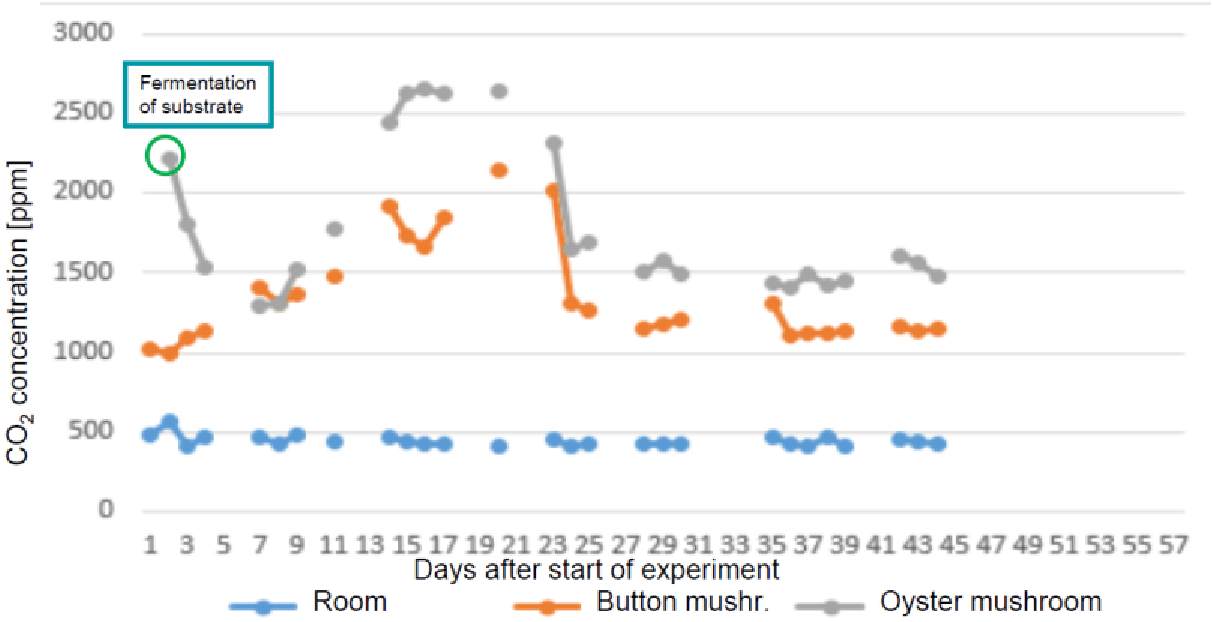

Figure 10 CO2 emission by button and oyster mushrooms

Measured in a 400 litre growth chamber.

In the preliminary experiment it was realised that the mushroom cultivation by the end of the experiment increased the CO_2 levels by *c.* 500 ppm (button mushrooms) or *c.* 1000 ppm (oyster mushrooms). To achieve a comparable CO_2 level in the larger cabin 4 by the mushroom cultivation, it was calculated that 212.5 (= 85/0.4) substrate bags with mushroom would be necessary. Consequently, with 50 substrate bags the CO_2 increase within cabin 4 should be in the range of 117 (button mushrooms) to 235 ppm (oyster mushrooms).

The strategy to enrich the CO_2 concentration by leading CO_2 from a mushroom cultivation into the greenhouse cabin at BOKU was successful and enabled to increase CO_2 levels on average by 127-156 µmol mol^{-1} (30 – 35 %) depending on mushroom activity (Tab. 2). The slightly lower than expected CO_2 for the selected oyster mushrooms may be explained by the different source of the mushrooms (Pilzmännchen vs. Hut & Stiel) and the different cultivation conditions.

In the experiment with mushroom cultivation, it was decided to inactivate the cabin temperature regulation of cabin 4 up to *c.* 12.00 – 13.00 o'clock in order to keep the atmospheric CO_2 levels in cabin 4 elevated as long as possible. This resulted in a small but distinct rise of mean temperature in the corresponding cabin by 3-5 °C during the light period, but not during the dark period. This temperature difference could have influenced the experimental results.

Table 2 Mean CO2 concentration in cabins with mushroom cultivation

Significances are based on Paired T-Tests & Bonferroni correction. Different letters in a row indicate significant differences at the 5% probability level.

Period considered	C3 (shading) [ppm]	C4 (shading & CO_2) [ppm]	C5 (control) [ppm]	Crop
30/01/-07/04/2025	446 ± 30 b	592 ± 99 a	431 ± 25 c	*Ocimum basilicum* L. 'Hohes Grünes Basilikum'

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28/02/-15/04/2025	431 ± 19 b	574 ± 90 a	419 ± 11 b	*Raphanus sativus* L. cv. Riesen von Aspern

The experiments with the combination of elevated atmospheric CO_2 concentrations by mushroom cultivation and with or without additional light revealed in the case of basil and radish unequivocally that sufficient light intensity is of fundamental importance and cannot be compensated for by additional CO_2. Only in the case a “certain” light level is achieved, adding CO_2 may have a positive effect on photosynthesis, yield and also water use efficiency.

The cultivation conditions within the cabins of the BOKU greenhouse are characterized by generally low light intensities due to the urban environment and are specific to this location. As a consequence, the low light intensity became the limiting factor under shading conditions by nets or PV cells. It resulted in the fact that yield was generally highest in the control group. Consequently, it is summarised that the supply of CO_2Bags or the addition of extra CO_2 by mushroom cultivation in order to maintain distinctly elevated atmospheric CO_2 levels in the chambers were able to compensate for only some of the negative effects of the limiting light availability. Especially carbohydrate content (of leaves) tended to be elevated in some experiments, but ultimately elevated CO_2 levels were unable to completely substitute the required light intensity at the BOKU experimental greenhouse.

Chapter 5 – CO_2 Enrichment at UTH

CO_2 enrichment installation

Carbon dioxide (CO_2) enrichment was implemented at the UTH greenhouse facilities in Greece during the first two experimental periods, as permitted by the weather conditions prevailing in Greece. For this purpose, twelve pressurized metal CO_2 cylinders were rented from SOL S.A. (Montza, Italy) exclusively for the duration of the experiments. Each cylinder contained liquefied CO_2 stored at approximately 250 bars, with a total combined weight of 400 kg. The CO_2 cylinders were installed outside the greenhouse to ensure operational safety. Prior to injection into the greenhouse compartments, the CO_2 passed through a pressure reduction system, with calibrated output 1 atm., that allowed the transition from liquid to gaseous form. A heating unit was integrated into the system to stabilize the gas temperature before distribution. CO_2 was delivered into the greenhouse through perforated distribution tubes installed beneath each hydroponic channel, enabling uniform gas dispersion within the treated compartments. CO_2 enrichment was applied in two out of four greenhouse compartments (Compartments 1 and 3), while the remaining compartments served as non-enriched controls.

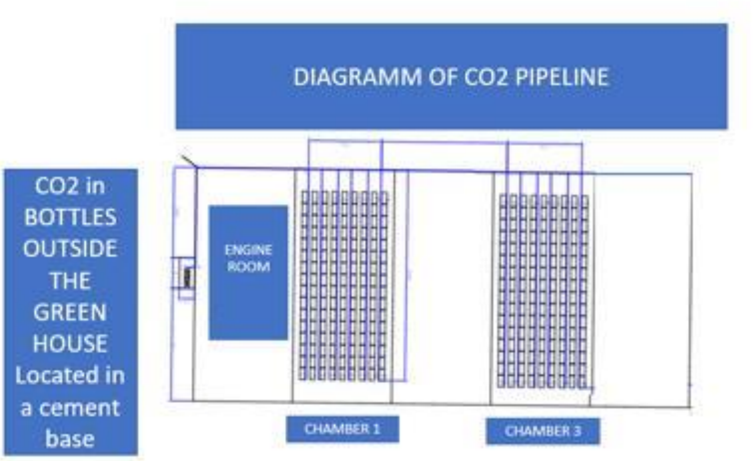

Figure 11 Illustration of CO2 pipeline installation

Greenhouse climate control and monitoring

Greenhouse climate conditions were continuously monitored and regulated using an automated climate-control computer (SERCOM, Automation SL, Lisse, The Netherlands). Each greenhouse compartment was equipped with a meteorological station positioned at the center of the compartment. Sensors measured air temperature (°C), relative humidity (RH, %), and CO_2 concentration (ppm), all installed at a height of 1.5 m above ground level. The target CO_2 concentration during enrichment periods was set at 600 ppm. The enrichment was initiated when ambient solar radiation exceeded 100 W m^{-2}. The duration of the enrichment was set to start from 8:00 am to 15:30 pm. The climate-control system ensured stable environmental conditions across all treatments, minimizing potential confounding effects of temperature and humidity fluctuations during enrichment events.

Experimental design and treatments

Four experimental treatments were established as shown in Figure 12:

1. Control (CTRL): No photovoltaic (PV) modules and no CO_2 enrichment
2. CO_2 enrichment (CO_2): CO_2 enrichment only
3. Photovoltaic tracking (PV-Tracking): Solar-tracking PV modules only
4. Combined treatment (PV-Tracking + CO_2): Solar-tracking PV modules with simultaneous CO_2 enrichment

Figure 12 CO2 cylinders placed outside the greenhouse

CO_2 enrichment was applied in Experiments A and B. In Experiment A, enrichment started on the 62nd day after transplantation (DAT) and ended on DAT 91. In Experiment B, enrichment began at DAT 0 and continued until DAT 55. Experiment B was conducted from January 16 to April 3, 2025, following the same experimental design as Experiment A.

CO_2 concentration results

In total, approximately 850 kg CO_2 were consumed through the two experimental trials. Mean CO_2 concentrations during the full cultivation cycles were generally comparable among treatments, including those with CO_2 enrichment (Table 3). However, during active enrichment periods, CO_2 -enriched compartments consistently exhibited higher CO_2 concentrations compared to non-enriched compartments. During enrichment events, average CO_2 concentrations in non-enriched treatments ranged between 355 and 399 ppm, while enriched compartments maintained significantly elevated levels, ranging from approximately 454 to 478 ppm. These values correspond to an increase of about 100 ppm relative to non-enriched compartments, demonstrating the effectiveness of the CO_2 enrichment system. The elevated CO_2 levels were directly attributable to controlled CO_2 release and confirmed the proper functioning of the installation and regulation system.

Table 3 Mean CO2 concentration

(ppm, ± S.E.) during the experimental periods, and average CO_2 concentration (ppm, ± SE), during the CO_2 enrichment events in fertilized and non-fertilized treatments.

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	Treatments	CO_2 [ppm]	CO_2 during enrichment [ppm]
Exp. A	Ambient air	331.5±0.30	–
	CTRL	389.3±0.89	372.1±3.09
	CO_2	400.7±0.48	454.1±5.95
	PV-Tracking	423.2±0.44	399.1±3.16
	PV-Tracking + CO_2	361.4±1.11	471.5±7.51
Exp. B	Ambient air	356.4±0.33	–
	CTRL	337.1±0.59	355.2±0.84
	CO_2	422.1±1.15	457.6±3.61
	PV-Tracking	412.2±0.44	386.9±0.71
	PV-Tracking + CO_2	431.1±1.21	478.8±3.56

Diurnal CO_2 concentration patterns revealed distinct responses during enrichment days (Figure 13). Although the set point was 600 ppm, CO_2 concentrations in enriched compartments continued to increase briefly after valve closure, indicating residual accumulation within the greenhouse air volume. Following the end of the programmed enrichment period (15:30), CO_2 concentrations declined rapidly, reflecting enhanced plant assimilation.

In the absence of air renewal, CO_2 concentrations temporarily dropped below ambient levels, particularly in previously enriched compartments. During night time hours, CO_2 concentrations in all treatments exceeded ambient air levels due to plant respiration. Notably, enriched compartments exhibited lower CO_2 concentrations than the control during evening hours without enrichment, suggesting increased photosynthetic capacity and CO_2 uptake by plants previously exposed to elevated CO_2.

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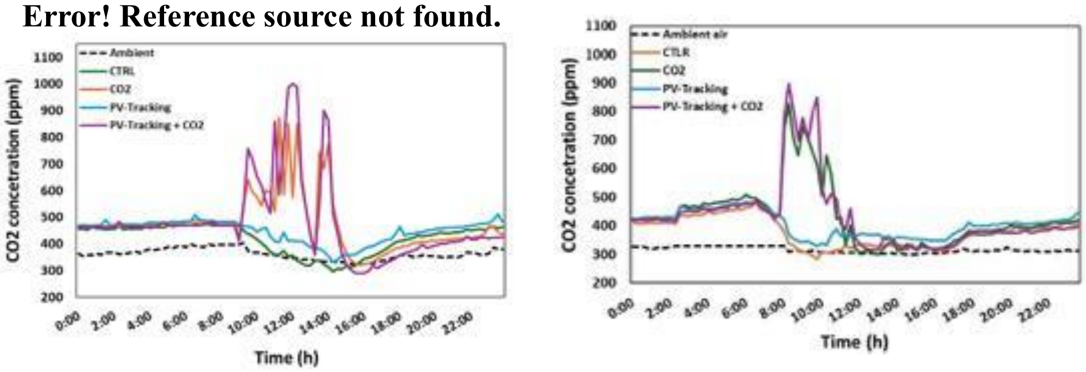

(A) **(B)**

Figure 13 Diurnal variation of CO2 concentrations

In control and enriched compartments across an experimental day (Experiments A and B).

Despite the known physiological effects of elevated CO_2 on stomatal conductance and transpiration, no significant differences in air temperature or relative humidity were observed between treatments during CO_2 enrichment periods. Mean temperature and RH values remained comparable across all treatments in both Experiments A and B.

Table 4 Mean air temperature

(°C, ± S.E) and relative humidity (%, ± S.E.) during CO_2 fertilization events in the first and second experimental periods.

	Treatments	Temperature [°C]	RH [%]
Exp. A	CTRL	18.3±0.16	87.6±0.71
	CO_2	18.2±0.17	88.0±0.72
	PV-Tracking	18.4±0.17	86.1±0.73
	PV-Tracking +CO_2	18.4±0.16	84.9±0.72
Exp. B	CTRL	20.2±0.09	68.8±0.26
	CO_2	20.2±0.09	67.5±0.26
	PV-Tracking	20.5±0.09	65.1±0.27
	PV-Tracking +CO_2	20.4±0.08	66.1±0.27

This indicates that the automated climate-control system successfully maintained stable greenhouse microclimatic conditions, ensuring that observed differences in CO_2 concentration were not accompanied by unintended changes in temperature or humidity.
Across all experimental periods, total yield did not differ significantly among treatments (Table 5). In Experiments A and B, CO_2-enriched treatments exhibited a non-significant trend toward higher productivity. In Experiment A, fresh biomass production was similar across treatments, although the PV-Tracking treatment exhibited a slight reduction in dry biomass. In Experiment B, plants under the CO_2 treatment produced the lowest fresh biomass, but this difference was not significant when expressed as dry biomass.

Table 5 Mean total fresh biomass

(g plant^{-1}), dry biomass (g plant^{-1}), and total yield (kg m^{-2}) (± SE) across treatments during the first, second, and third experimental periods.

	Treatments	Fresh biomass [g plant^{-1}]	Dry biomass [g plant^{-1}]	Total yield [kg m^{-2}]
Exp. A	CTRL	988.0±47.36 a	117.6±2.99 a	8.62±0.29 a
	CO_2	1092.0±30.58 a	119.4±3.80 a	8.92±0.43 a
	PV-Tracking	1008.6±17.85 a	108.2±1.09 b	8.30±0.20 a
	PV-Tracking+CO_2	1028.0±13.78 a	114.4±1.24 ab	8.89±0.72 a
Exp. B	CTRL	811.6±41.63 a	61.10±2.37 a	5.12±0.22 a
	CO_2	703.8±9.98 b	59.98±0.60 a	5.18±0.32 a
	PV-Tracking	769.4±31.70 ab	62.03±2.66 a	5.15±0.14 a
	PV-Tracking+CO_2	791.8±45.17 ab	66.18±4.08 a	5.68±0.25 a

Chapter 6 – CO_2 Enrichment at Alzahrawy

The CO_2 enrichment system at Alzahrawy in Israel uses technical-grade CO_2 supplied from cylinder bundles as its primary source. Each cylinder bundle has a capacity of approximately 50 kilograms of CO_2, and multiple bundles are available, resulting in a maximum total on-site capacity of about 300 to 350 kilograms of CO_2.

The greenhouse area currently equipped with a closed and controlled CO_2 enrichment environment covers approximately 160 square meters.

CO_2 input is monitored through flow measurements taken at the pressure regulator and by manual readings. In addition, continuous monitoring data are provided by CO_2 sensors, allowing the total quantity of CO_2 used to be measured and logged.

The typical duration of CO_2 enrichment is on average two to four hours each morning, depending on ventilation requirements and the internal greenhouse temperature. During summer days, CO_2 enrichment is often not used at all due to excessively high temperatures.

The target CO_2 concentration during periods of active photosynthesis is between 700 and 900 parts per million (ppm). These values are monitored either manually or automatically.

In practice, stable CO_2 concentrations of 550 to 750 ppm are achieved before ventilation begins. Under fully closed conditions, concentrations of up to 800 ppm can be reached briefly. However, during ventilation, CO_2 levels typically decrease to between 450 and 550 ppm.

These data are consistent with other observations, which indicate that in Israel, effective CO_2 enrichment is largely limited to the early morning hours due to high temperatures and the associated need for continuous ventilation.

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