agronomy

Article

Effects of Elevated CO_2 Levels on the Growth and Yield of Summer-Grown Cucumbers Cultivated under Different Day and Night Temperatures

Hiromi Namizaki [1], Yasunaga Iwasaki [2] and Rui Wang [3,*]

[1] Kumamoto Prefecture Central Administrative Headquarters, Agriculture and Forestry Department, Kumamoto 861-1113, Japan
[2] Agricultural Department, Meiji University, Kurokawa 2060-1, Aso-ku, Kawasaki 101-0062, Japan
[3] Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, 3-1-1, Kannondai, Tsukuba 305-8519, Japan
* Correspondence: ruiwangsyau@126.com

Abstract: The effects of elevated CO_2 (eCO_2) levels on field-grown cucumbers have been extensively studied. However, the variations in photosynthate accumulation in summer-grown cucumbers simultaneously exposed to eCO_2 and varying day-night temperatures (DNF) still remain unexplored. This study aimed to investigate the effects of DNF different CO_2 conditions [ambient CO_2 (aCO_2; 400–600 μmol mol^{-1}) and eCO_2 (800–1000 μmol mol^{-1})] on dry matter production and dry matter distribution in summer-grown cucumbers under two DNF treatments (35/10 °C and 25/20 °C, day/night). We observed that long-term eCO_2 exposure increased C assimilation and photosynthate accumulation in leaves, resulting in feedback inhibition of the leaf area. Under both DNF treatments, the total dry matter distribution to fruits under eCO_2 conditions was approximately 15% higher than that under aCO_2 conditions. Furthermore, soluble sugar content and C:N ratio increased with long-term eCO_2 exposure, indicating increased C allocation, photosynthate accumulation, and distribution. However, low night temperatures (LT) inhibited respiration and increased dry matter accumulation by 30% under eCO_2 conditions. Additionally, eCO_2 increased fruit fresh weight by 8% and 12% under both DNF treatments compared to aCO_2. This suggests that long-term eCO_2 exposure and varying DNF exhibited different effects through different metabolic mechanisms on cucumber growth at high temperatures. eCO_2 conditions probably increased dry matter distribution to improve fruit quality, and LT treatment altered the respiration rate to restore photosynthesis, thereby increasing photosynthate distribution to fruits. Therefore, a combination of CO_2 enrichment and DNF can be used to improve fruit quality and yield at high temperatures.

Keywords: carbon assimilation; day-night temperature; dry matter distribution; growth; photosynthate accumulation; greenhouse

Citation: Namizaki, H.; Iwasaki, Y.; Wang, R. Effects of Elevated CO_2 Levels on the Growth and Yield of Summer-Grown Cucumbers Cultivated under Different Day and Night Temperatures. *Agronomy* **2022**, *12*, 1872. https://doi.org/10.3390/agronomy12081872

Academic Editors: Jean-Claude Roy, Thierry Boulard and Shumei Zhao

Received: 13 July 2022
Accepted: 6 August 2022
Published: 9 August 2022

1. Introduction

The cucumber is one of the most important vegetable crops which is cultivated year-round in commercial greenhouses. However, in the summer, temperatures above the optimal range notably affect field conditions, which ultimately decrease fruit quality and yield [1–4]. To address this issue, greenhouse conditions, including average daily (ADT), day (DT), and night (NT) temperatures and the DNF difference between DT and NT (DNF) for the whole cultivation period, are highly regulated. Previous studies have indicated that both DNF and ADT affect the morphology and developmental rate of the cucumber, including internode length, dry matter content, number of flower buds, and secondary metabolism, as well as the field conditions [5–7]. Furthermore, the cucumber growth rate is more highly affected by ADT than DT or NT individually because an increase in DT enhances dry weight more than a similar increase in NT [5]. During flower development, an increase in negative DNF values considerably decreases the number of flower buds compared with an increase in positive DNF values. ADT and DNF also exhibit different effects on cucumber yield; ADT alters the maturation time and yield, whereas DNF improves fruit quality [8,9]. Therefore, ADT and DNF are important determinants of plant growth, especially in fruit and vegetable species in which temperature variations alter development at different stages.

Additionally, elevated CO_2 (eCO_2) enhances the photosynthetic rate and boosts whole-canopy photosynthesis. eCO_2 also increases leaf area, dry matter content, foliar C:N ratio, source–sink conditions, fruit quality, and yield [10–12]. Therefore, CO_2 enrichment in the horticultural industry has received a great deal of attention over the past years. Willits and PEET proved the effect of CO_2 on the enrichment time and concentration on the yield of cucumbers and tomatoes and suggested that the optimum concentration is inversely related to the length of the enrichment period and enrichment hours [13]. Other researchers focused on the mean and long-term evaluation of CO_2 and determined the effect of CO_2 on the fruit biomass even in the low-radiation conditions [14].

Nonetheless, photosynthate accumulation varies with CO_2 levels, exposure time, and temperature. Short-term CO_2 exposure (few days) increases the photosynthetic rate, whereas the massive photosynthate accumulation under long-term CO_2 enrichment (few weeks to months) results from the negative feedback on photosynthesis, which decreases the photosynthetic rate and acclimation [15–17]. Furthermore, eCO_2 improves C assimilation rates in the leaf, which further increases photosynthetic acclimation.

Simultaneously, abnormal accumulation of soluble carbohydrates and starch results in photosynthetic acclimation in fruits [18]. However, massive starch accumulation in leaves creates a pressure gradient between the leaves and roots, which, in turn, promotes the distribution of soluble carbohydrates [19]. Moreover, under eCO_2 conditions, plants exhibit high sink–source and flow–source ratios of photosynthetic assimilative C abundance [13]. This suggests that under CO_2 enrichment, increasing the K content improves photosynthate distribution from the source (leaf) to the flow (stem) and sink (root) in cucumber plants. eCO_2 probably regulates fertilizer assimilation and improves resistance and fruit quality under stress and varying climatic conditions [20,21]. Previous studies have also demonstrated the effects of eCO_2 levels on the interactions between CO_2 and other environmental conditions, including light intensity, water-use efficiency, N management, and temperature [22].

The cucumber is highly sensitive to high temperatures, suggesting that the effects of eCO_2, particularly in the flowering and fruiting stages, can vary throughout its growing period during summer [23,24]. However, limited information is available on the combined effects of DNF and eCO_2 levels on photosynthate accumulation and C allocation to different organs in summer-grown cucumbers.

Here, we investigated the long-term effects of eCO_2 levels on the growth of and C partitioning in greenhouse-grown cucumbers. To this aim, we examined sink-organ growth, fruit yield, and C:N ratios of cucumbers grown at two NTs (high and low) under ambient CO_2 (aCO_2) or eCO_2 conditions. This study will provide insights into the growth response of the cucumber to the combination of eCO_2 and DNF and C flow in different plant organs, which will be beneficial for the management of summer-grown cucumbers in commercial greenhouses.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiments were conducted in four greenhouses (floor area: 6.0 m^2) located at the Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, Japan (36.04° N, 140.03° E). Meteorological data, including solar radiation, air temperature, CO_2 level, and humidity were recorded using a data logger (GL-1000; Graphtech, Yokohama, Japan) at 10-min intervals. Cucumber seeds, obtained from Greenway (Saitama Gensyu Ikuseikai Co., Ltd., Saitama, Japan), were sown on 5 May and transplanted on 2 June on rockwool slab (Grodan Expert, Grodan BV, Roermond, The Netherlands) on an elevated bench (0.5 m above the ground) placed at the center of each greenhouse. The distance between two plants was 0.18 m. The growing beds were oriented in the north–south direction with every side shoot pinched at two nodes, and old leaves were pruned every week. A nutrient solution (Otsuka House Solution S1; Otsuka Agritechno Co., Ltd., Tokyo, Japan), with an electrical conductivity of 0.8–1.2 dS m^{-1}, was supplied to the growing beds using a drip system. The nutrient of OH-A are N (260 g m^{-3}), NH_4-N (23 g m^{-3}), NO_3-N (233 g m^{-3}), P_2O_5 (120 g m^{-3}), K_2O (405 g m^{-3}), CaO (230 g m^{-3}), MgO (60 g m^{-3}), MnO (1.5 g m^{-3}), B_2O_3 (1.3 g m^{-3}), Fe (2.7 g m^{-3}), Cu (0.03g m^{-3}), Zn (0.09 g m^{-3}), and Mo (0.03 g m^{-3}).

2.2. Experimental Design and Treatments

Greenhouses maintained low (LT; 35/10 ± 2 °C, day/night) and high (HT; 25/20 ± 2 °C, day/night) NTs using cooling systems that comprised 1.0 m3 water tanks. The water temperature in the cooling systems was maintained at approximately 10 °C using heat pumps (UWYP125A; Daikin Co., Ltd., Tokyo, Japan). The average day and night relative humidities (RH) were 75 ± 0.1% and 80 ± 0.3%, respectively. CO_2 levels in the greenhouses were monitored using CO_2 concentration sensors (CO_2 engine K-30; Sense Air Co., Ltd.,Tokyo, Japan. Plants grown in each greenhouse were subjected to aCO_2 (400–600 µmol mol^{-1}) and eCO_2 (HC, 800–1000 µmol mol^{-1}) conditions. The four treatments are shown in Figures 1 and 2.

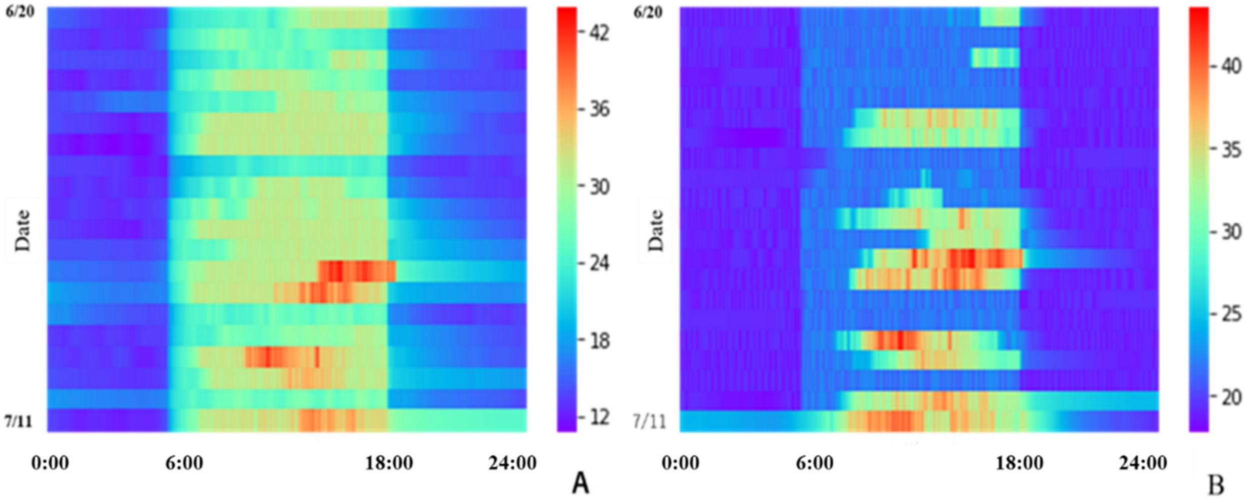


Figure 1. Temperature variations in (**A**) low (LT, 35/10 ± 2 °C, day/night) and (**B**) high (HT, 25/20 ± 2 °C, day/night) night temperature (NT) treatments.

2.3. Growth Parameters

The experimental design in the study is randomized completed block design. To estimate the growth and development, six plants were randomly selected from each treatment for non-destructive measurements at six sampling times: 14/6, 16/6, 23/6, 30/6, 7/7, and 15/7, that is, 14, 21, 28, 35, and 43 d after transplantation, respectively. For these plants, leaf number and length at each node, total number of leaves, stem length, fruit number, and number of branches were determined every week. Destructive measurements were recorded on 1 and 15 July. Mean values of leaf area and fresh and dry weights of plant organs were determined using four replicates. Leaf area was measured using a leaf area meter (LI-3100A; Lincoln Co., Ltd., Lincoln, NE, USA). Then, leaves, stems, and fruits were oven-dried at 80 °C for 48 h to measure their dry weights.

Yield was determined using the number and fresh and dry weights of whole and standard fruits. Thereafter, total C and N content in 10 mg dry powder were measured to determine the C:N ratio using the Pregl–Dumas method and a CN coder (Jm-1000; J-Science, Tokyo, Japan).

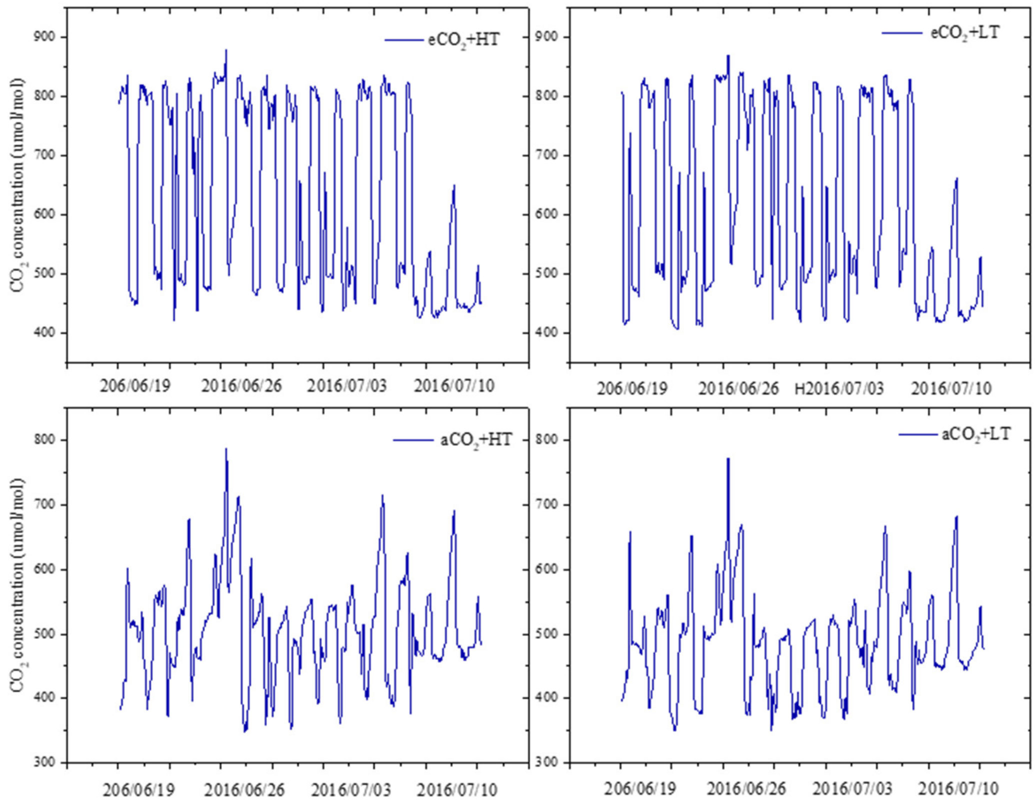


Figure 2. Variations in daily CO_2 levels under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments.

Subsequently, soluble sugar and starch content were determined according to Nakano et al. (1995) [25]. Destructively sampled leaves and stems were oven-dried at 80 °C for at least a week, weighed, and ground. Sucrose was extracted using 80% (v/v) ethanol at 80 °C, and its concentration in the supernatant was enzymatically determined using a test kit (No. 716260; R-Biopharm AG, Darmstadt, Germany). Starch was extracted from the precipitate, and the concentrations were enzymatically determined using another test kit (No. 207748; R-Biopharm AG), following the manufacturer's instructions and a standard regression plot.

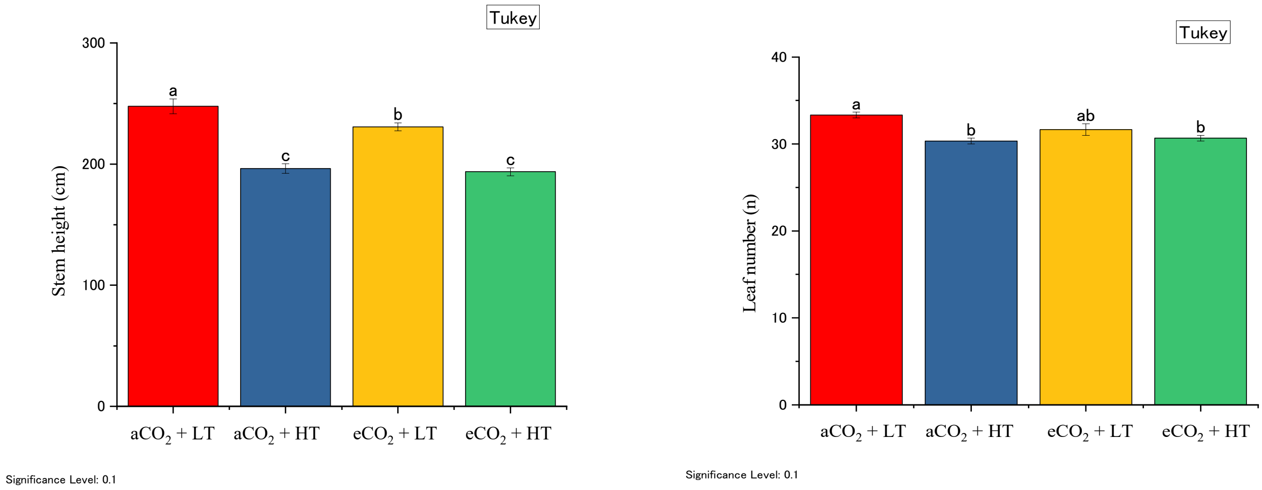
2.4. Statistical Analyses

Statistical analyses were performed using the software Origin (Origin 2021b, OriginLab Corporation, Northampton, UK). Data were first normalized and transformed and then subjected to analysis of variance (ANOVA) to determine the effects of CO_2 and DNF treatments on plant growth. Significant differences among treatments were determined using Tukey–Kramer's multiple comparison test ($p < 0.005$).

3. Results

3.1. Effects of CO_2 and DNF Treatments on Morphological Parameters and Dry Matter

The morphological characteristics of the cucumber plants cultivated under different CO_2 and DNF treatments are shown in Figure 3. eCO_2 exhibited varying effects on cucumber growth and leaf number under LT and HT. In the plants exposed to HT, stem height decreased by 15% after 43 d of eCO_2 exposure; however, eCO_2 exhibited no effect on the plants exposed to LT (Figure 3A). Furthermore, stems were significantly taller (20%) in plants exposed to HT than those exposed to LT (Figure 3A). Compared to the HT treatment, the leaves appeared approximately 7% earlier than those in the LT treatment. Nonetheless, no difference in leaf number was observed in plants exposed to the LT treatment (Figure 3B), indicating that LT treatment inhibited the effects of eCO_2 over long cultivation periods.



(A) **(B)**

Figure 3. (**A**) Stem height and (**B**) leaf number at 14, 21, 28, 35, and 43 d after transplantation under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Vertical bars represent standard error (SE) of mean ($n = 10$). For all variables with the same letter, the difference between the means is not statistically significant. If two variables have different letters, they are significantly different.

Owing to eCO_2 levels throughout the growth period, notable differences in dry matter accumulation were observed in plants exposed to different DNF treatments (Figure 4). In the LT treatment, although eCO_2 did not affect leaf dry weight, it increased the total dry weight by 30% (Figure 4). In contrast, eCO_2 increased leaf dry weight by 28% without altering the total dry weight of the plants exposed to HT (Figure 4). Moreover, the eCO_2 and DNF treatments exhibited long-term effects on dry matter accumulation compared with morphological characteristics (Figures 3 and 4). However, variations in dry matter accumulation were not recorded until 20 d after transplantation (Figure 4).

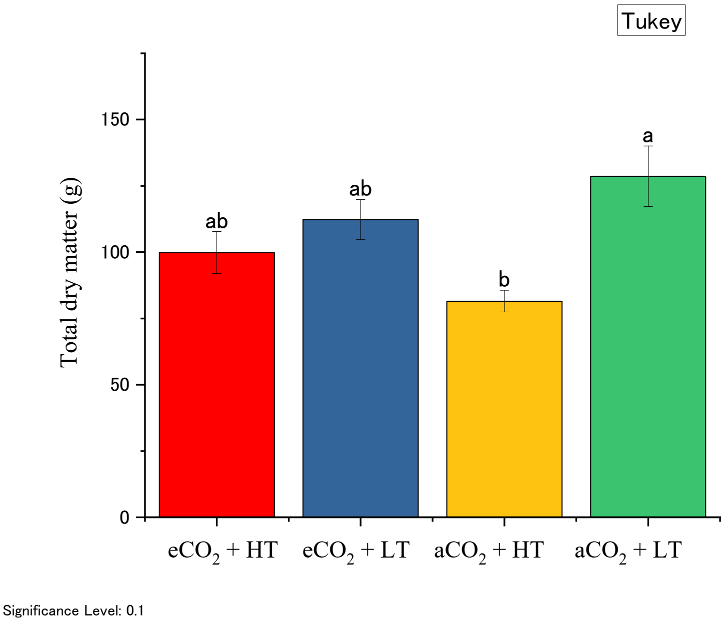


Figure 4. Total and leaf dry matter at 14, 28, and 43 d after transplantation under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Vertical bars represent SE of mean ($n = 10$). Error bars indicate SE of mean. Different lowercase letters indicate significant differences among treatments ($p < 0.005$).

3.2. Growth Rate and Photosynthesis

To determine the growth responses under the eCO_2 and DNF treatments, we measured stem length, total leaf number and area, number and weight of fruits and branches, and number of leaves on branches at harvesting (Table 1). HT markedly increased stem (14.8%) and internode length, irrespective of CO_2 levels. However, branch number increased by 20% under the eCO_2 + LT treatment.

Table 1. Analysis of variance (ANOVA) of morphological parameters at harvesting under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Data represent mean ± SE (n = 6).

CO_2 Treatment	Temperature Treatment	Stem Length (cm)	Internode Length (cm)	Leaf Number	Fruit Number	Fruit Weight (g)	Branch Number	Weight of Branch (g)	Leaf Number of Branch	Total Leaf Area (cm^2)
eCO_2	LT	193.67 ± 5.69^{b}	6.32 ± 0.13^{b}	30.67 ± 0.58^{a}	16.50 ± 2.10^{a}	2071 ± 182^{b}	10.7 ± 0.58^{a}	45.47 ± 7.51^{a}	14.67 ± 2.31^{b}	$11{,}188.01 \pm 1412.00^{a}$
	HT	230.67 ± 5.77^{a}	7.29 ± 0.24^{a}	31.67 ± 0.53^{a}	16.50 ± 1.70^{a}	1958 ± 170^{a}	9.3 ± 0.58^{b}	49.67 ± 11.80^{b}	14.00 ± 1.73^{a}	$10{,}919.81 \pm 1370.93^{b}$
a CO_2	LT	196.33 ± 6.81^{b}	6.47133 ± 0.11^{b}	30.33 ± 0.58^{a}	15.2 ± 1.60^{a}	1867 ± 208^{a}	9.0 ± 1.58^{a}	36.87 ± 10.51^{b}	12.00 ± 4.00^{a}	$10{,}187.84 \pm 1238.68^{ab}$
	HT	247.67 ± 6.59^{a}	7.4281 ± 0.20^{a}	33.33 ± 0.58^{a}	15.3 ± 1.90^{a}	1662 ± 181^{b}	12.3 ± 1.28^{b}	48.73 ± 2.93^{a}	19.67 ± 5.51^{b}	$16{,}604.81 \pm 5283.47^{b}$
ρ-value										
CO_2		n.s.	n.s.	**	**	**	*	*	*	**
Temperature		***	***	**	**	**	*	*	*	**
Interaction		***	***	**	***	***	*	*	*	**

Note: Different lower-case letters in the same column indicate a significant difference among treatments (LSD multiple range test, $p < 0.05$, n.s.: non-significant DNFferenc, *: significant at $p < 0.05$, **: significant at $p < 0.01$, ***: significant at $p < 0.001$).

The interaction between the eCO_2 and DNF treatments significantly affected the fruit number and weight at harvesting. eCO_2 significantly improved fruit weigh under the LT treatment. Fruit weight under the eCO_2 + LT treatment also increased by 19.7% compared with that under the aCO_2 + HT treatment. Under eCO_2 levels, LT increased fruit weight by 5.2% compared with HT. Nonetheless, the difference in fruit weight between LT and HT treatments was 10.9% under aCO_2 levels.

The absolute (AGR) and relative growth rates (RGR), net assimilation rate (NAR), leaf area ratio (LAR; lamina area per unit plant weight), and specific leaf area (SLA; ratio of leaf area to its dry weight) of the cucumber plants exposed to the four treatments are shown in Table 1.

Similar to previous studies, eCO_2 levels increased plant growth rate. AGR under eCO_2 + LT, eCO_2 + HT, aCO_2 + LT, and aCO_2 + HT treatments varied from 60.958 g d^{-1} to 127.335 g d^{-1}, 53.152 g d^{-1} to 145.530 g d^{-1}, 46.412 g d^{-1} to 102.841 g d^{-1}, and 44.631 g d^{-1} to 120.634 g d^{-1}, respectively. Similar results were observed for RGR, indicating increased carbohydrate accumulation, which favored cucumber growth. Furthermore, NAR was significantly altered by DNF treatments; LT increased NAR by 11.67% compared with HT. However, increased dry matter accumulation did not increase LAR; LAR under eCO_2 + LT, eCO_2 + HT, aCO_2 + LT, and aCO_2 + HT treatments varied from 0.012 m^2 g^{-1} to 0.005 m^2 g^{-1}, 0.010 m^2 g^{-1} to 0.006 m^2 g^{-1}, 0.014 m^2 g^{-1} to 0.006 m^2 g^{-1}, and 0.014 m^2 g^{-1} to 0.006 m^2 g^{-1}, respectively. Nonetheless, eCO_2 significantly decreased (15%) SLA compared with aCO_2 under both DNF treatments.

3.3. Synergistic Effects of eCO_2 and DNF Treatments on Fruit Yield

We observed the accumulated fresh weight significantly varied from day 10 to the beginning of harvesting (Figure 5). Polynomial fitting curves of variations in fresh weight suggested that eCO_2 significantly increased fresh weight from the beginning to the end of harvesting under both DNF treatments. Furthermore, the increase in fresh weight in LT-treated plants accelerated the increase in accumulated fresh weight. The accumulated fresh weights exceeded 3000 g and 2500 g in the eCO_2 + LT and eCO_2 + HT treatments, respectively, and were approximately 2000 g and 1500 g in the aCO_2 + LT and aCO_2 + HT treatments, respectively. Therefore, the eCO_2 + LT treatment significantly increased dry matter accumulation in fruits.

Dry matter distribution in plants was determined on days 0, 14, and 29 after transplantation (Figure 6). Dry matter distribution varied during long-term eCO_2 exposure. Initially, the leaf dry weight was 5.4 g in all the treatments, approximately 80% of the total dry matter. On day 29 after transplantation, differences in fruit weight were observed between eCO_2

and aCO_2 treatments. Under eCO_2 conditions, fruit dry weights were 97.6 g and 99.6 g in the LT and HT treatments, respectively, whereas it was 88.9 g and 80.1 g in the LT and HT treatments, respectively, under the aCO_2 conditions. Increased dry matter accumulation in eCO_2-treated plants can be attributed to increased photosynthesis. Interestingly, in the long-term eCO_2 treatments, more dry matter was distributed to the fruits than the leaves. Leaf dry weight per plant under eCO_2 + LT, eCO_2 + HT, aCO_2 + LT, and aCO_2 + HT treatments were 81.0 g, 72.5 g, 89.5 g, and 55.5 g, respectively.

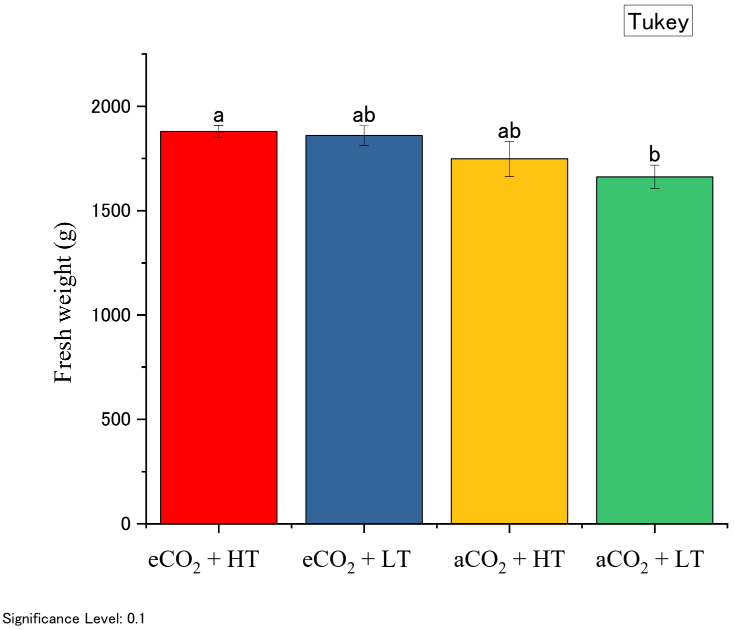


Figure 5. Fruit fresh weight at different harvesting dates under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments (n = 6). Error bars indicate SE of mean. Different lowercase letters indicate significant differences among treatments ($p < 0.005$).

Both average fruit number and fresh weight per plant did not significantly increase upon eCO_2 treatment (Table 2). However, eCO_2 exhibited significant effects on fruit quality and yield (Table 2). The standard fruit number per plant (16.3 ± 0.5 and 16.8 ± 1.5 for LT and HT treatments, respectively) and fresh weight (1882.5 ± 50 g and 1998.3 ± 70 g for LT and HT treatments, respectively) increased by approximately 10% upon eCO_2 exposure compared to aCO_2 treatment.

Table 2. ANOVA of absolute (AGR) and relative growth rates (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments during two periods (16/6–1/7 and 1/7–15/7). Data represent mean ± SE (n = 6).

CO_2 Treatment	**Temperature Treatment**	**AGR**		**RGR**		**NAR**		**LAR**		**SLA**	
		($g\ d^{-1}$)		**($g\ g^{-1}\ d^{-1}$)**		**($g\ m^2\ d^{-1}$)**		**($m^2\ g^{-1}$)**		**($m^2\ g^{-1}$)**	
		Periods		**Periods**		**Periods**		**Periods**		**Periods**	
		6/16–7/1	7/1–7/15	6/16–7/1	7/1–7/15	6/16–7/1	7/1–7/15	6/16–7/1	7/1–7/15	6/16–7/1	7/1–7/15
eCO_2	LT	60.958^{a}	127.335^{b}	0.149^{a}	0.075^{bc}	12.649^{a}	10.332^{c}	0.012^{b}	0.005^{b}	0.018^{c}	0.013^{d}
	HT	53.152^{b}	145.53^{a}	0.141^{b}	0.088^{b}	10.694^{b}	11.701^{b}	0.01^{c}	0.006^{a}	0.014^{d}	0.015^{c}
a CO_2	LT	46.412^{c}	102.841^{d}	0.133^{c}	0.105^{a}	9.113^{c}	12.498^{a}	0.014^{a}	0.006^{a}	0.022^{a}	0.017^{b}
	HT	44.631^{c}	120.634^{c}	0.131^{c}	0.086^{b}	8.987^{d}	8.627^{d}	0.014^{a}	0.006^{a}	0.021^{b}	0.018^{a}
ρ-value											
CO_2		**	***	**	**	**	***	**	***	**	***
Temperature		n.s.	**	n.s.	**	**	***	n.s.	n.s.	**	**
Interaction		**	**	**	**	***	***	**	**	**	***

Note: Different lower-case letters in the same column indicate a significant difference among treatments (LSD multiple range test, $p < 0.05$, n.s.: non-significant Differenc, **: significant at $p < 0.01$, ***: significant at $p < 0.001$).

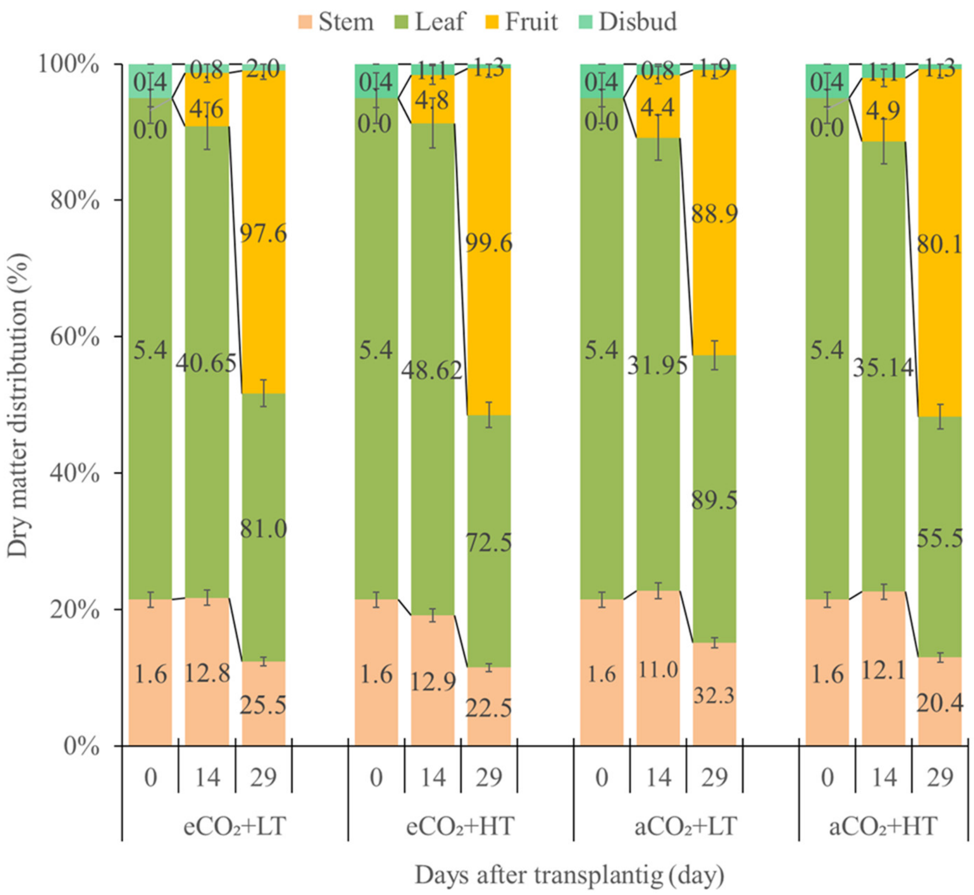


Figure 6. Dry matter distribution in different plant parts under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Number in columns represent dry weights of different plant parts. Error bars indicate SE of mean (*n* = 10).

3.4. *Effects of eCO$_2$ and DNF Treatments on Carbohydrate Content and C:N Ratio*

The effects of the eCO_2 and DNF treatments on developing (number: 11–15) and developed (number: 20–25) leaves are shown in Figure 7. We observed that soluble sugar and starch content varied between developing and developed leaves. Soluble sugar content was higher in developed leaves under the eCO_2 + HT treatment than in those exposed to other treatments. However, soluble sugar content did not vary in the developing leaves under different DNF treatments, except under the aCO_2 + HT treatment where the soluble sugar content slightly increased.

Furthermore, eCO_2 levels significantly affected starch content in both developing and developed leaves (Figure 7). Starch content significantly increased from 80 mg g^{-1} to 110 mg g^{-1} and 100 mg g^{-1} in plants exposed to eCO_2 + LT and eCO_2 + HT treatments. Although starch content in developed leaves increased from 60 mg g^{-1} to 80 mg g^{-1} under both DNF treatments, the differences were insignificant.

The C:N ratios in developing and developed leaves, stems, and fruits are shown in Figure 8. eCO_2 significantly affected the C:N ratios in leaves, stems, and fruits. The C:N ratios of the developing leaves significantly varied between eCO_2 + LT and aCO_2 + LT treatments (Figure 8A). Moreover, eCO_2 + LT treatment significantly increased the C:N ratio of the developed leaves by 24.1% (Figure 8B). Furthermore, the C:N ratios of developed leaves (Figure 8B) and stem (Figure 8C) exhibited similar patterns; the highest C:N ratio was observed in the eCO_2 + LT treatment (16.3 in developed leaves and 22.1 in stem), whereas the lowest C:N ratio was recorded in the aCO_2 + LT treatment (11 in developed leaves and 16.2 in stem). Nonetheless, DNF treatment exhibited no significant effect on the C:N ratio of fruits under the eCO_2 conditions, as shown Figure 8D.

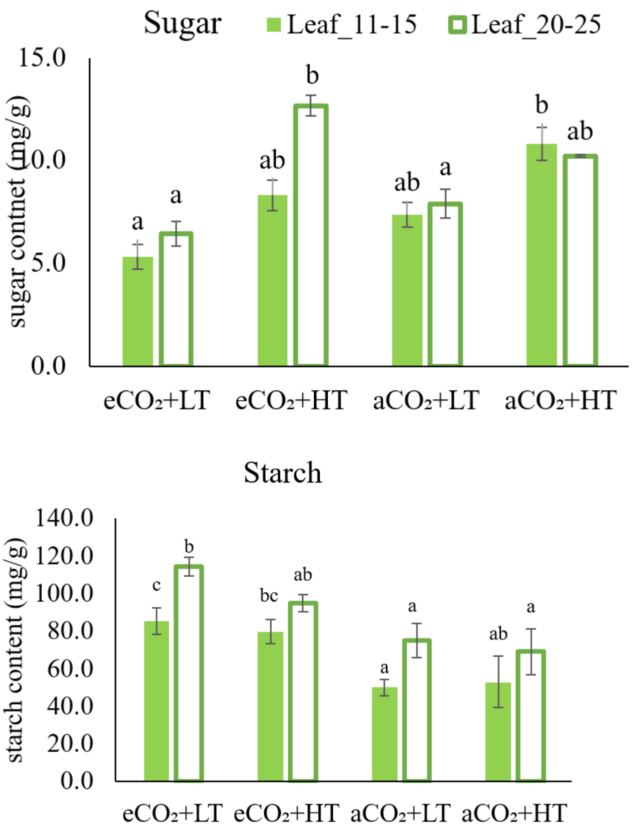


Figure 7. Soluble sugar and starch content in developing (numbers: 11–15) and developed (numbers: 20–25) leaves of plants under aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Error bars indicate SE of mean ($n = 3$). Different lowercase letters indicate significant differences among treatments ($p < 0.005$).

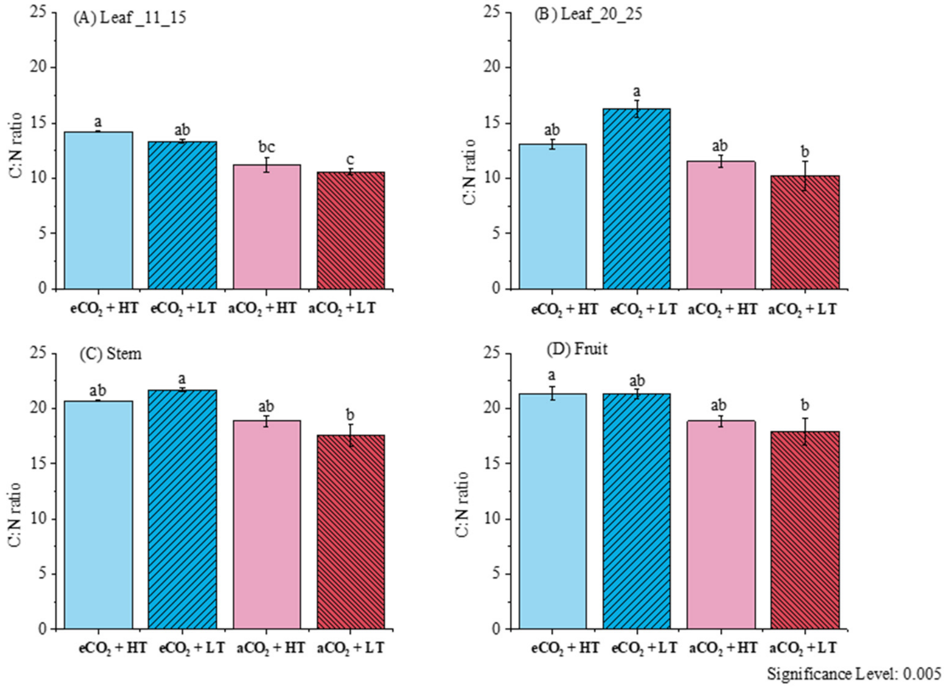


Figure 8. Average C:N ratios of (**A**) developing leaves, (**B**) developed leaves, (**C**) stem, and (**D**) fruit at the end of aCO_2 + LT, aCO_2 + HT, eCO_2 + LT, and eCO_2 + HT treatments. Error bars indicate SE of mean. Different lowercase letters indicate significant differences among treatments ($p < 0.005$).

Additionally, there were no significant differences in the C:N ratios exposed to the DNF treatments. However, eCO_2 altered the fruit C:N ratios (Figure 8D); the fruit C:N ratios increased by 10.2% and 19.0% in the eCO_2 + LT and eCO_2 + HT treatments, respectively, and the highest fruit C:N ratio (21.5) was observed under the eCO_2 + LT treatment.

4. Discussion

4.1. eCO_2 and DNF Treatments Affect Photosynthesis, Growth, and Dry Matter Content

Previous studies suggest that eCO_2 levels affect plant physiology, growth, and productivity [26]. eCO_2 promotes the accumulation of secondary metabolites, modulates secondary metabolism, improves adaptability, photosynthesis, and net assimilation capacity, thereby increasing crop yield [18,27,28]. In addition, few studies have demonstrated the effects of combinations of eCO_2 levels and other environmental factors, including treatment time (short- and long-term), temperature, light intensity, and water availability, on the growth of several crop varieties [3,22,26]. Nonetheless, the long-term effects of the eCO_2 and DNF treatments on plants cultivated at high temperatures still remain unelucidated.

Few studies have verified that eCO_2 levels or high temperatures significantly increase dry matter content, which can be further enhanced using a combination of high temperature and CO_2 enrichment [29]. However, contrasting results have been reported in other plant species. Klopotek and Kläring [30] reported that dry matter content was significantly higher when tomato plants (*Solanum lycopersicum* L.) were grown at low temperatures. Lee et al. [26] suggested that plant phenology was increasingly affected by high temperatures rather than eCO_2 levels at all growth stages, and elevated temperatures strongly influenced dry matter production in annual grasses during the reproductive phase compared to the vegetative phase.

We observed the long-time photosynthetic responses to eCO_2 levels and DNF treatments in this study. It can be found that DNF affected production of photosynthesis, such as stem growth, dry matter distribution, and leaf development, at high temperatures. The leaves play a role in photosynthesis, which uses light energy to produce carbohydrates from the atmospheric CO_2. We also observed taller plants with more leaves in the aCO_2 + HT treatment compared to the aCO_2 + LT treatment (Figure 1). Moreover, plants exposed to the aCO_2 + LT treatment exhibited higher total and leaf dry matter than those exposed to the aCO_2 + HT treatment (Figure 2). Owing to the high day temperature, photosynthate accumulation was slower in plants exposed to the aCO_2 + LT treatment than those exposed to the aCO_2 + HT treatment. These results indicate that high temperatures promoted dry matter production and inhibited respiration at night during summer. Compared with aCO_2, eCO_2 increased dry matter distribution, which was significantly enhanced under Different NT. The highest dry matter content was observed in plants exposed to the eCO_2 + LT treatment. These findings are consistent with previous reports on cotton leaves where eCO_2 levels or high temperatures increased dry matter content [31–33].

On the hand, the effects of CO_2 enrichment on photosynthate accumulation were observed, such as stem height and leaf number were insignificant in plants exposed to the aCO_2 + LT treatment (Figure 1). We also analyzed variations of photosynthate accumulation by the ANOVA of absolute (AGR) and relative growth rates (RGR), net assimilation rate (NAR), and leaf area ratio (LAR), as shown in Table 2. These results indicated that during summer, the impact of DNF on photosynthate accumulation was more significant than that of CO_2 enrichment. Moreover, dry matter accumulation was evident 20 d after transplantation in all treatments. Therefore, we hypothesized that the sink organ or capacity was not sufficiently strong enough to consume or mobilize carbohydrates during early growth stages. Hence, there was no sink limitation at the beginning of the eCO_2 treatment.

4.2. eCO_2 and DNF Treatments Affects Dry Matter Partition and Fruit Yield

The long-term effects of eCO_2 on photosynthetic acclimation can be attributed to various reasons, including the inhibition of protein synthesis, N partitioning, C:N ratio, and sink strength [30–34]. C sink strength is a key limiting factor for plant yield, as plants exposed to eCO_2 levels have limited C sink strength and exhibit decreased photosynthetic rates to stabilize C source activity and sink capacity [35–38].

In this study, leaf area and total dry matter increased upon eCO_2 treatment, and dry matter distribution to each plant part varied with the growth stage. Although photosynthate accumulation increased in plants exposed to eCO_2 levels, their leaf area decreased; the total leaf area of plants exposed to the eCO_2 + LT and eCO_2 + HT treatments were not significantly larger than those exposed to the aCO_2 + LT and aCO_2 + HT treatments.

The findings of this study indirectly verified the downregulation of photosynthetic acclimation in new organs, including branches and fruits, under eCO_2 levels (Table 1). During fruit formation, eCO_2 promoted dry matter distribution to fruits compared to aCO_2 (Figure 6). These results are consistent with previous reports on the effects of eCO_2, wherein photosynthate accumulation increased in leaves, and excess dry matter altered photosynthesis, resulting in its translocation to fruits and other tissues [39].

eCO_2 levels affect plant physiology and biochemistry by altering primary and secondary metabolism, including alterations in biomass, nutrients, functional components, and hardness [40–42]. Although eCO_2 has been employed to enhance photosynthesis and crop yield, it may deteriorate the nutritional quality of crops, including lettuce, spinach, and tomato (lycopene content) [43]. In this study, we observed that eCO_2 increased the fresh weight of standard cucumber fruits compared with those grown under the aCO_2 conditions; however, the increase in fruit number was insignificant (Table 2). The increase in fruit quality under eCO_2 levels was consistent with the report by Zhang et al. (2017), which suggested that eCO_2 decreased the percentage of small fruits in tomato plants cultivated under water-limited conditions [7].

A previous study reported that DNF alone exhibited no effect on maturation time and yield; however, it improved the percentage of first-grade cucumbers [6]. In this study, a higher fruit number was observed in plants exposed to the LT treatment, irrespective of the CO_2 level. This indicates that fruit number was closely associated with DNF. Nonetheless, the effects of DNF on the number of standard fruit and yield were insignificant; the number of standard fruits per plant was 15 for both aCO_2 + LT and aCO_2 + HT treatments. eCO_2 levels improved fruit quality in both DNF treatments, and the numbers of standard fruits per plant were 16.3 and 16.8 in the eCO_2 + LT and eCO_2 + HT treatments, respectively.

4.3. eCO_2 and DNF Treatments Affects C Flow and C:N Ratios

Alterations in the content of soluble sugar and starch indicate the effects of eCO_2 and varying temperatures on the photosynthetic machinery [44]. Several studies have reported a direct correlation between photosynthetic acclimation to eCO_2 levels and variations in leaf carbohydrate content [45,46]. This study suggested that the increased content of soluble sugar and starch and decreased Rubisco content might be partially responsible for photosynthetic downregulation upon eCO_2 exposure. These findings are consistent with previous reports.

Furthermore, we observed that the content of soluble sugars and starch increased in both developing and developed leaves under long-term eCO_2 exposure (Figure 7). Compared with developed leaves, developing leaves accumulated more soluble sugars and starch, which can be attributed to the sensitivity of developing leaves to eCO_2 and high temperatures. These findings are consistent with previous studies [45] which suggest that young leaves are highly sensitive to the environment and resource availability. It also showed that the sugar accumulation in fruit depends on the accumulation of photosynthesis.

This study reports the effects of combined exposure to DNF and eCO_2 at high temperatures. In this study, sugar content was more strongly correlated with DNF than with eCO_2. The highest carbohydrate content (12.7 mg g^{-1}) was observed in developed leaves exposed to the eCO_2 + HT treatment, which was much higher than that observed in the developed leaves exposed to the eCO_2 + LT treatment (6.4 mg g^{-1}) (Figure 7). Moreover, starch content was highly affected by the eCO_2 levels than the DNF treatments; the starch content in plants exposed to eCO_2 was much higher than those exposed to aCO_2, irrespective of the DNF treatment. These results indirectly verify that eCO_2 mitigates the adverse effects of high temperatures [7].

The C flow from the leaves supports roots and young organs, such as buds, flowers, and fruits, via the phloem. It acts as limiting factors for plant growth and crop yield and plays an important role in N metabolic processes, such as nitrate reduction and assimilation to support other organs. Under the eCO_2 and DNF treatments (Figure 6), it also indicates that photosynthetic carbon loss can be reduced by using the eCO_2 and DNF treatments.

C and N are essential for metabolism, and their bioavailability is tightly coordinated for optimal plant growth and development. The majority of photosynthates formed during leaf C assimilation are destined for respiration, storage, or export to other tissues [1,46]. The leaf is the primary C source organ, which acquires C from dry matter to form a C pool under eCO_2 conditions. Therefore, the leaf dry matter was higher in plants exposed to long-term eCO_2 conditions compared with those exposed to aCO_2 conditions. These findings suggest that the LT treatment limited respiration and resulted in the storage of a significant proportion of the C pool in the leaves, which decreased leaf sink strength and hampered carbohydrate assimilation. As shown in Table 2, the RGR of cucumber plants exposed to eCO_2 levels did not increase, suggesting that the C sink strength of leaves remained unaltered upon long-term eCO_2 exposure. Therefore, photosynthesis was downregulated and photosynthates were distributed to other organs, including fruits. These findings are consistent with those of other studies where excess photosynthates were distributed to stem or roots under stress conditions [42,44]. In contrast, when NT was constant, photosynthates were utilized during respiration, thereby increasing the sink strength of recently fixed C, which resulted in reduced photosynthetic rate in leaves. This is especially important for plants cultivated under high temperatures and controlled environments of greenhouses. Thus, we confirmed the mean and long-term evaluation of CO_2 and determine the effect of CO_2 on the fruit biomass even in the high-temperature conditions. This means that the effect of eCO_2 on the production agrees well with previous results on the agricultural response of the cucumber.

Variations in the C:N ratio are important internal indicators of plant growth and provide feedback on temperature variations in controlled environments [10,45]. Several hypotheses have been proposed to explain the variations in C:N ratio. Rachmilevitch et al. [46] reported that eCO_2 probably inhibits photorespiration and N assimilation, thereby limiting the capacity of plants to translocate photosynthates from the leaves. Recently, Dong et al. [19] reported that the inhibition of N assimilation under eCO_2 conditions occurred prior to the decrease in sink strength. These findings also indicate that long-term exposure to eCO_2 inhibits N assimilation. The higher C:N ratio in plants exposed to eCO_2 (Figure 8) verified abundant photosynthate translocation to fruits and the variations in fruit C: N, which, in turn, indirectly affirmed the effects of eCO_2 levels on fruit quality [34]. These results also suggest that cucumber plants exhibit plasticity not only in morphology but also in physiological traits in response to different C availability.

5. Conclusions

This study suggests that long-term eCO_2 exposure and DNF treatments affect photosynthetic acclimation and yield in summer-grown cucumber at high temperatures. Compared with aCO_2, eCO_2 increased dry matter accumulation. Furthermore, the DNF treatment significantly increased the effects of CO_2 enrichment by inhibiting respiration at night. We also observed the downregulation of photosynthate distribution to organs other than leaves, including branches and fruits, under eCO_2 conditions. Moreover, we observed increased photosynthetic distribution to fruits and variations in the fruit C:N ratio. These findings suggest that although eCO_2 levels increased the fresh weight of standard fruits compared to aCO_2, no significant increase was observed in the number of whole fruits. Therefore, cucumber plants exhibited plasticity in morphology, as well as physiological traits, in response to C availability and high temperatures.

Author Contributions: H.N. and Y.I. designed the study and carried out the experiment. R.W. wrote the manuscript with support from Y.I. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data presented in this study are available on fair request to the corresponding authors.

Acknowledgments: We wish to thank all our colleagues for their assistance in the research for this paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hrichi, S.; Chaabane-Banaoues, R.; Bayar, S.; Flamini, G.; Oulad El Majdoub, Y.; Mangraviti, D.; Mondello, L.; El Mzoughi, R.; Babba, H.; Mighri, Z.; et al. Botanical and Genetic Identification Followed by Investigation of Chemical Composition and Biological Activities on the *Scabiosa atropurpurea* L. Stem from Tunisian Flora. *Molecules* **2020**, *25*, 5032. [CrossRef] [PubMed]
2. Ali, M.; Ayyub, C.M.; Amjad, M.; Ahmad, R. Evaluation of thermo-tolerance potential in cucumber genotypes under heat stress. *Pak. J. Agric. Sci.* **2019**, *56*, 53–61.
3. Wang, L.; Feng, Z.; Schjoerring, J.K. Effects of elevated atmospheric CO_2 on physiology and yield of wheat (*Triticum aestivum* L.): A meta-analytic test of current hypotheses. *Agric. Ecosyst. Environ.* **2013**, *178*, 57–63. [CrossRef]
4. Hikosaka, S.; Sugiyama, N. Effects of Exogenous Plant Growth Regulators on Yield, Fruit Growth, and Concentration of Endogenous Hormones in Gynoecious Parthenocarpic Cucumber (*Cucumis sativus* L.). *Hortic. J.* **2015**, *84*, 342–349. [CrossRef]
5. Dan, K.; Imada, S. Effect of High Temperature on Viability and Growth of Radicles in Cucumber Seedlings. *J. Jpn. Soc. Hortic. Sci.* **2002**, *71*, 805–811. [CrossRef]
6. Grimstad, S.O.; Frimanslund, E. Effect of different day and night temperature regimes on greenhouse cucumber young plant production, flower bud formation and early yield. *Sci. Hortic.* **1993**, *53*, 191–204. [CrossRef]
7. Guo, R.; Li, X.; Christie, P.; Chen, Q.; Zhang, F. Seasonal temperatures have more influence than nitrogen fertilizer rates on cucumber yield and nitrogen uptake in a double cropping system. *Environ. Pollut.* **2008**, *151*, 443–451. [CrossRef]
8. Sato, S.; Kamiyama, M.; Iwata, T.; Makita, N.; Furukawa, H.; Ikeda, H. Moderate increase of mean daily temperature adversely affects fruit set of Lycopersicon esculentum by disrupting specific physiological processes in male reproductive development. *Ann. Bot.* **2006**, *97*, 731–738. [CrossRef]
9. Springer, C.J.; Ward, J.K. Flowering time and elevated atmospheric CO_2. *New Phytol.* **2007**, *176*, 243–255. [CrossRef]
10. Carolina, S.; Iker, A.; Inmaculada, P.; Gorka, E.; Álvaro, S.; Jone, A.; Manuel, S.; Juan, J.; José, L.; Fermín, M. Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. *J. Plant Physiol.* **2015**, *174*, 97–109. [CrossRef]
11. Vanuytrecht, E.; Raes, D.; Willems, P. Considering sink strength to model crop production under elevated atmospheric CO_2. *Agric. For. Meteorol.* **2011**, *151*, 1753–1762. [CrossRef]
12. Likun, L.; Mengfei, W.; Sabin, S.P.; Chunxu Li Megha NParajulee Fajun, C.; Wanping, F. Effects of elevated CO_2 on foliar soluble nutrients and functional components of tea, and population dynamics of tea aphid. *Plant Physiol. Bio-Chem.* **2019**, *145*, 84–94. [CrossRef]
13. Willits, D.H.; Peet, M.M. Predicting yield responses to different greenhouse CO_2 enrichment schemes: Cucumbers and tomatoes. *Agric. For. Meteorol.* **1989**, *44*, 275–293. [CrossRef]
14. Sa´nchez-Guerrero, M.C.; Lorenzo, P.; Medrano, E.; Castilla, N.; Soriano, T.; Baille, A. Effect of variable CO2 enrich-ment on greenhouse production in mild winter climates. *Agric. For. Met.* **2005**, *132*, 244–252. [CrossRef]
15. Cho, A.R.; Song, S.J.; Chung, S.W.; Kim, Y.J. CO_2 Enrichment with Higher Light Level Improves Flowering Quality of Phalaenopsis Queen Beer 'Mantefon'. *Sci. Hortic.* **2018**, *247*, 356–361. [CrossRef]
16. Pérez-López, U.; Sgherri, C.; Miranda-Apodaca, J.; Micaelli, F.; Lacuesta, M.; Mena-Petite, A.; Quartacci, M.F.; Muñoz-Rueda, A. Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO_2. *Plant Physiol. Biochem.* **2018**, *123*, 233–241. [CrossRef]
17. Sui, X.; Shan, N.; Hu, L.; Zhang, C.; Yu, C.; Ren, H.; Turgeon, R.; Zhang, Z. The complex character of photosynthesis in cucumber fruit. *J. Exp. Bot.* **2017**, *68*, 1625–1637. [CrossRef]
18. Dabu, X.; Li, S.; Cai, Z.; Ge, T.; Hai, M. The effect of potassium on photosynthetic acclimation in cucumber during CO_2 enrichment. *Photosynthetica* **2019**, *57*, 640–645. [CrossRef]
19. Dong, J.; Li, X.; Chu, W.; Duan, Z. High nitrate supply promotes nitrate assimilation and alleviates photosynthetic acclimation of cucumber plants under elevated CO_2. *Sci. Hortic.* **2017**, *218*, 275–283. [CrossRef]

20. Farfan-Vignolo, E.R.; Asard, H. Effect of elevated CO_2 and temperature on the oxidative stress response to drought in *Lolium perenne* L. and *Medicago sativa* L. *Plant Physiol. Biochem.* **2012**, *59*, 55–62. [CrossRef]
21. Yang, X.; Zhang, P.; Wei, Z.; Liu, J.; Hu, X.; Liu, F. Effects of CO_2 fertilization on tomato fruit quality under reduced irrigation. *Agric. Water Manag.* **2019**, *230*, 105985. [CrossRef]
22. Oh-E, I.; Saitoh, K.; Kuroda, T. Effects of High Temperature on Growth, Yield and Dry-Matter Production of Rice Grown in the Paddy Field. *Plant Prod. Sci.* **2007**, *10*, 412–422. [CrossRef]
23. Overdieck, D.; Fenselau, K. Elevated CO_2 concentration and temperature effects on the partitioning of chemical components along juvenile Scots pinestems (*Pinus sylvestris* L.). *Trees* **2009**, *23*, 771–786. [CrossRef]
24. Nakano, H.; Makino, A.; Mae, T. Effects of Panicle Removal on the Photosynthetic Characteristics of the Flag Leaf of Rice Plants during the Ripening Stage. *Plant Cell Physiol.* **1995**, *36*, 653–659. [CrossRef]
25. Lee, J.-S. Combined effect of elevated CO_2 and temperature on the growth and phenology of two annual C3 and C4 weedy species. *Agric. Ecosyst. Environ.* **2011**, *140*, 484–491. [CrossRef]
26. Seneweera, S.; Makino, A.; Hirotsu, N.; Norton, R.; Suzuki, Y. New insight into photosynthetic acclimation to elevated CO_2: The role of leaf nitrogen and ribulose-1,5-bisphosphate carboxylase/oxygenase content in rice leaves. *Environ. Exp. Bot.* **2011**, *71*, 128–136. [CrossRef]
27. Izargi, V.; Daniel, M.; Joseba, S.; Carmen, G.; Jose María, E.; María, B. CO_2 enrichment modulates ammonium nutrition in tomato adjusting carbon and nitrogen metabolism to stomatal conductance. *Plant Sci.* **2015**, *241*, 32–44. [CrossRef]
28. Zhang, S.; Fu, W.; Zhang, Z.; Fan, Y.; Liu, T. Effects of elevated CO_2 concentration and temperature on some physiological characteristics of cotton (*Gossypium hirsutum* L.) leaves. *Environ. Exp. Bot.* **2017**, *133*, 108–117. [CrossRef]
29. Aranjuelo, I.; Irigoyen, J.J.; Perez, P.; Martinez-Carrasco, R.; Sanchez-Diaz, M. Response of nodulated alfalfa to water supply, temperature and elevated CO_2: Productivity and water relations. *Environ. Exp. Bot.* **2006**, *55*, 130–141. [CrossRef]
30. Huang, G.; Rymer, P.D.; Duan, H.; Smith, R.A.; Tissue, D.T. Elevated temperature is more effective than elevated [CO_2] in exposing genotypic variation in Telopea speciosissima growth plasticity, implications for woody plant populations under climate change. *Glob. Chang. Biol.* **2015**, *21*, 3800–3813. [CrossRef]
31. Klopotek, Y.; Kläring, H.-P. Accumulation and remobilisation of sugar and starch in the leaves of young tomato plants in response to temperature. *Sci. Hortic.* **2014**, *180*, 262–267. [CrossRef]
32. Aranjuelo, I.; Irigoyen, J.; Sánchez-Díaz, M.; Nogués, S. Carbon partitioning in N_2 fixing Medicago sativa plants exposed to different CO_2 and temperature conditions. *Funct. Plant Biol.* **2008**, *35*, 306–317. [CrossRef] [PubMed]
33. Aranjuelo, I.; Pardo, A.; Biel, C.; Savé, R.; Azcón-Bieto, J.; Nogués, S. Leaf carbon management in slow-growing plants exposed to elevated CO_2. *Glob. Change Biol.* **2009**, *15*, 97–109. [CrossRef]
34. Moutinho-Pereira, J.M.; Bacelar, E.; Gonçalves, B.; Ferreira, H.M.F.; Coutinho, J.; Correia, C.M. Effects of Open-Top Chambers on physiological and yield attributes of field grown grapevines. *Acta Physiol. Plant.* **2010**, *32*, 395–403. [CrossRef]
35. Giri, A.; Armstrong, B.; Rajashekar, C.B. Elevated Carbon Dioxide Level Suppresses Nutritional Quality of Lettuce and Spinach. *Am. J. Plant Sci.* **2016**, *7*, 246–258. [CrossRef]
36. Zahara, M.; Datta, A.; Boonkorkaew, P. Effects of sucrose, carrot juice and culture media on growth and net CO_2 exchange rate in Phalaenopsis hybrid 'Pink'. *Sci. Hortic.* **2016**, *205*, 17–24. [CrossRef]
37. Moore, B.D.; Cheng, S.-H.; Sims, D.; Seemann, J.R. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant Cell Environ.* **1999**, *22*, 567–582. [CrossRef]
38. Reyes, T.H.; Scartazza, A.; Lu, Y.; Yamaguchi, J.; Guglielminetti, L. Effect of carbon/nitrogen ratio on carbohydrate metabolism and light energy dissipation mechanisms in Arabidopsis thaliana. *Plant Physiol. Biochem.* **2016**, *105*, 195–202. [CrossRef]
39. Iván, J.; Pedro, M.A.; Concepción, A.; Marina, R.; Iker, A. Root and shoot performance of Arabidopsis thaliana exposed to elevated CO_2: A physiologic, metabolic and transcriptomic response. *J. Plant Physiol.* **2015**, *189*, 65–76. [CrossRef]
40. Chen, C.-T.; Setter, T.L. Response of potato dry matter assimilation and partitioning to elevated CO_2 at various stages of tuber initiation and growth. *Environ. Exp. Bot.* **2012**, *80*, 27–34. [CrossRef]
41. Peltonen, P.A.; Vapaavuori, E.; Julkunen-Tiitto, R. Accumulation of phenolic compounds in birch leaves is changed by elevated carbon dioxide and ozone. *Global. Chang. Biol.* **2005**, *11*, 1305–1324. [CrossRef]
42. Peter, B.; Michael, T.; Rebecca, F.; Audrey, L.; Glenn, J.F.; Malcolm, J.H.; Sabine, T. Expression patterns of C- and N-metabolism related genes in wheat are changed during senescence under elevated CO_2 in dry-land agriculture. *Plant Sci.* **2015**, *236*, 239–249. [CrossRef]
43. Jefferson, R.S.; Angelica, E.P.; Weverton, P.R.; Eliemar, C.; Kevin, L.G. Photosynthetic acclimation to elevated CO_2 combined with partial root zone drying results in improved water use efficiency, drought tolerance and leaf carbon balance of grapevines (*Vitis labrusca*). *Environ. Exp. Bot.* **2017**, *134*, 82–95. [CrossRef]
44. Royer, M.; Larbat, R.; Le Bot, J.; Adamowicz, S.; Robin, C. Is the C:N ratio a reliable indicator of C allocation to primary and defence-related metabolisms in tomato? *Phytochemistry* **2013**, *88*, 25–33. [CrossRef]
45. Wubs, A.M.; Ma, Y.T.; Heuvelink, E.; Hemerik, L.; Marcelis, L.F.M. Model selection for nondestructive quantification of fruit growth in pepper. *J. Am. Soc. Hortic. Sci.* **2012**, *137*, 71–79. [CrossRef]
46. Rachmilevitch, S.; Asaph, B.C.; Arnold, J.B. Nitrate assimilation in plant shoots depends on photorespiration. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11506–11510. [CrossRef]