Root-Zone CO2 Concentration Affects Partitioning and Assimilation of Carbon in Oriental Melon Seedlings

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Abstract: Root-zone CO2 is essential for plant growth and metabolism. However, the partitioning and assimilation processes of CO2 absorbed by roots remain unclear in various parts of the oriental melon. We investigated the time at which root-zone CO2 enters the oriental melon root system, and its distribution in different parts of the plant, using 13C stable isotopic tracer experiments, as well as the effects of high root-zone CO2 on leaf carbon assimilation-related enzyme activities and gene expressions under 0.2%, 0.5% and 1% root-zone CO2 concentrations. The results showed that oriental melon roots could absorb CO2 and transport it quickly to the stems and leaves. The distribution of 13C in roots, stems and leaves increased with an increase in the labeled root-zone CO2 concentration, and the δ13C values in roots, stems and leaves increased initially, and then decreased with an increase in feeding time, reaching a peak at 24 h after 13C isotope labeling. The total accumulation of 13C in plants under the 0.5% and 1% 13CO2 concentrations was lower than that in the 0.2% 13CO2 treatment. However, the distributional proportion of 13C in leaves under 0.5% and 1% 13CO2 was significantly higher than that under the 0.2% CO2 concentration. Photosynthetic carbon assimilation-related enzyme activities and gene expressions in the leaves of oriental melon seedlings were inhibited after 9 days of high root-zone CO2 treatment. According to these results, oriental melon plants’ carbon distribution was affected by long-term high root-zone CO2, and reduced the carbon assimilation ability of the leaves. These findings provide a basis for the further quantification of the contribution of root-zone CO2 to plant communities in natural field conditions.

Keywords: root-zone CO2; oriental melon; 13C stable isotope tracing; carbon assimilation

1. Introduction

In agricultural production, improper irrigation, root respiration, microbial activities and the decomposition of various types of organic matter in the soil will lead to the enrichment of root-zone CO2 and a decrease in O2 content. This greatly impacts the growth and development of plants [1–6]. The rhizosphere has unique physicochemical and biological properties, which can regulate water absorption and nutrients and affect the reproduction of microorganisms [7–9]. Previous studies have shown that CO2 can be absorbed and fixed by roots, dissolved in the soil to form inorganic carbon, and then transported to stems and leaves to participate in photosynthesis and promote an increase in the total carbon content of plants [10–13]. The source and destination of carbon in plants can be quickly transported in plants, but since it takes time for them to transport photosynthates from the stems to the roots, more time is required to allocate 13C to the roots than to the stems and leaves [18,19]. CO2 is transported from the roots to the stems and leaves, where 13CO2...
flows out of the leaf surface and diffuses into the atmosphere [20,21]. The $^{13}$C tracer of Camptotheca acuminate seedlings showed that the soluble inorganic carbon absorbed by roots could be used as a carbon source for photosynthesis, affecting the formation of photosynthates [22].

Carbon assimilation is an enzymatic reaction that involves a variety of enzymes. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the key enzyme in photosynthesis, and the main limiting factor regarding photosynthetic CO$_2$ assimilation in C$_3$ plants. Many factors affect its activity [23,24]. Rubisco activating enzyme (RCA) activity can determine the Rubisco carboxylation efficiency and limit plant photosynthesis [25]. Transketolase (TK) was found to be involved in photosynthetic carbon fixation, and its activity significantly affects the photosynthetic rate [26]. Fructose-1,6-diphosphate esterase (FBPase) is an essential enzyme whose activity directly impacts carbohydrate accumulation and photosynthetic efficiency [27]. The regeneration of ribulose-1,5-diphosphate (RuBP) caused by sedoheptulose-1,7-bisphosphatase (SBPase) regulates the inflow of carbon [28]. Phosphoglycerate kinase (PGK) is highly conserved, and is involved in glycolysis and photosynthesis during photosynthetic carbon fixation [29]. Phosphoribulokinase (PRK) is a vital enzyme in the Calvin cycle that is involved in photosynthesis [30]. The gene expressions of carbon assimilation-related enzymes will affect related enzyme activities, thus affecting photosynthesis. Studies have shown that transgenic modified tobacco plants overexpress photosynthetic carbon assimilated FBPase, SBPase and inorganic carbon transporter B (ict B), and photosynthesis in these plants was significantly enhanced [31].

The oriental melon (Cucumis melo var. makuwa Makino) is very sensitive to rhizosphere gas. Previous studies have found that rhizosphere gas often affects plant growth and fruit quality in facility cultivation [32,33]. The present study aimed to reveal the distribution of carbon absorbed by roots in plants, and the changes in enzyme activities and gene expression related to carbon assimilation under the conditions of elevated carbon dioxide in the root zone of oriental melon seedlings. We utilized $^{13}$C stable isotope labeling technology in order to explore the effects of high root-zone CO$_2$ on the carbon absorption and carbon assimilation in oriental melon. The study provides a theoretical reference for further investigations into the response mechanisms of oriental melon root to high root-zone CO$_2$ and the regulation of the rhizosphere gas environment.

2. Results

2.1. Root-Zone $^{13}$CO$_2$ Concentration Affects the Abundance of $^{13}$C in Different Positions of Oriental Melon Plants

The $\delta^{13}$C of labeled ($^{13}$C-0.2%, $^{13}$C-0.5% and $^{13}$C-1%) treatments at L1, L2 and L3 increased with the extension of feeding time (Figure 1). The $\delta^{13}$C of L1 with labeled treatments was significantly higher than that of unlabeled (C-0.2%, C-0.5% and C-1%) treatments after 0.5 h. This can be explained by the fact that $^{13}$C was detected in L1 of the labeled treatment. In L2, the $\delta^{13}$C of $^{13}$C-0.5% and $^{13}$C-1% treatments were significantly higher than those of unlabeled treatments at 0.5 h, i.e., $^{13}$C was detected, while the $\delta^{13}$C of the $^{13}$C-0.2% treatment was significantly higher than that of unlabeled treatments at 1.5 h, and the detection time of $^{13}$C was later than that of $^{13}$C-0.5% and $^{13}$C-1% treatments. In L1 and L2, the $\delta^{13}$C of $^{13}$C-0.5% and $^{13}$C-1% labeled treatments were significantly higher than that of the $^{13}$C-0.2% labeled treatment, and the differences were enhanced with the extension of the feeding time. This shows that the greater the label concentration, the faster the transportation from root to shoot. At the L3 site, 1.5 h after feeding, the $\delta^{13}$C value of labeled treatments was significantly higher than that of unlabeled treatments, and the $^{13}$C-0.5% and $^{13}$C-1% treatments were significantly higher than that of $^{13}$C-0.2%, while $^{13}$C-1% treatment was significantly higher than that of $^{13}$C-0.5%. The results showed that oriental melon roots could absorb CO$_2$ and rapidly transport it upward; moreover, the higher the root-zone CO$_2$ concentration, the more CO$_2$ is absorbed by roots, and the faster the transportation speed to the aboveground region.
High root-zone $^{13}$CO$_2$ affects the abundance of $^{13}$C in roots and different aboveground nodes of oriental melon plants. L1 represents the roots, L2 represents the first and second real-leaf nodes of oriental melon plants. L1 represents the roots, L2 represents the first and second stems of the plant, and L3 represents other leaves and stems. The results showed that in 13C-0.5% and 13C-1% labeled treatments, the 13C distribution of 13C-0.5% and 13C-1% treatments was significantly higher than that of the 13C-0.2% treatment; moreover, the 13C-1% treatment was significantly higher than that of 13C-0.5%. The results showed that in 13C-0.5% and 13C-1% labeled treatments, the 13C distribution of 13C-0.5% and 13C-1% treatments was significantly higher than that of the 13C-0.2% treatment at 24 h and 72 h. It can be seen from Figure 2 that at 24 h and 72 h after labeling, the δ$^{13}$C values of 13C-0.2%, 13C-0.5% and 13C-1% treatments in roots, stems and leaves were significantly higher than those of unlabeled treatments (C-0.2%, C-0.5% and C-1%), and that there was no significant difference between unlabeled treatments; this indicates that 13C of labeled treatments in root-zone 13CO$_2$ could be detected in roots, stems and leaves. The δ$^{13}$C value in roots, stems and leaves showed an initial increase, followed by a decrease with the extension of the labeling time, and reached a peak at 24 h. The δ$^{13}$C values in roots, stems and leaves were significantly higher in the 13C-0.5% and 13C-1% treatments than that in the 13C-0.2% treatment; moreover, the 13C-1% treatment was significantly higher than that of 13C-0.5% at 24 h and 72 h, and the difference decreased with an increase in labeling time. The results showed that the root-zone CO$_2$ concentration and treatment time could affect the enrichment degree of root, stem and leaf to new carbon absorbed by the roots of oriental melon plants.
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Figure 2. High root-zone $^{13}$CO$_2$ affects the $^{13}$C abundance in roots, stems and leaves of oriental melon. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.

2.3. Root-Zone $^{13}$CO$_2$ Concentration Affects the Distribution of Carbon in Roots, Stems and Leaves of Oriental Melon

As can be seen from Figure 3, with the extension of treatment time, the $^{13}$C-0.2% treatment increased the $^{13}$C distribution in roots, stems and leaves, and the total amount of $^{13}$C in plants. The $^{13}$C distribution and total amount of $^{13}$C-0.5% and $^{13}$C-1% treatments increased initially and then decreased. The $^{13}$C distributions of $^{13}$C-0.5% and $^{13}$C-1% treatments were significantly higher than that of the $^{13}$C-0.2% treatment at 24 h and 72 h. Moreover, the $^{13}$C distribution of $^{13}$C-1% in roots, stems and leaves was significantly higher than that for $^{13}$C-0.5%. The results showed that in $^{13}$C-0.5% and $^{13}$C-1% labeled treatments, roots absorbed more carbon than in the $^{13}$C-0.2% treatment, and the $^{13}$C allocation increased before decreasing with the extension of the treatment time. The root-zone CO$_2$ concentration and treatment time affected the carbon allocation of each part of the plant.

Figure 3. High root-zone $^{13}$CO$_2$ affects the distribution of C in roots, stems and leaves of oriental melons. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.
2.4. Root-Zone $^{13}$CO$_2$ Concentration Affects the Distribution Proportion of Carbon in Roots, Stems and Leaves of Oriental Melon

The distribution ratio of labeled $^{13}$C in each part of the plant can indicate the distribution of carbon absorbed by the roots. It can be seen from Figure 4 that at 24 h and 72 h after labeling, the $^{13}$C distribution proportion of $^{13}$C-0.2% was significantly higher than that of the $^{13}$C-0.5% and $^{13}$C-1% treatments in stems and roots. Nevertheless, the distribution ratios of $^{13}$C in $^{13}$C-0.5% and $^{13}$C-1% treatments were higher than that of $^{13}$C-0.2% in leaves, and $^{13}$C-1% was significantly higher than $^{13}$C-0.5%. At 72 h after labeling, the distribution ratios of $^{13}$C-0.2%, $^{13}$C-0.5% and $^{13}$C-1% treatments were the highest in the stems and the lowest in the roots. With the extension of the feeding time, the distributional proportion of $^{13}$C in $^{13}$C-0.2%, $^{13}$C-0.5% and $^{13}$C-1% treatments decreased in roots but increased in stems; the $^{13}$C-0.2% treatment in leaves showed an increasing trend, while it showed a decreasing trend under $^{13}$C-1% treatment. The results showed that the distribution of CO$_2$ absorbed by roots in different parts of oriental melon was affected differently by the root-zone CO$_2$ concentration and treatment time: the higher the root-zone CO$_2$ concentration, the more significant the proportion of carbon distribution in aboveground leaves.

![Figure 4.](https://example.com/figure4.png)

2.5. Root-Zone $^{13}$CO$_2$ Concentration Affects the Accumulation of Biomass in Roots, Stems and Leaves of Oriental Melon

The results showed that the dry mass of roots, stems and leaves increased with an increase in treatment time, as shown in Figure 5. At 24 h after labeling, the difference in the dry mass of roots between the three CO$_2$ concentration labeling treatments was not significant; however, at 72 h after labeling, the $^{13}$C-0.5% and $^{13}$C-1% treatments yielded significantly lower values than the $^{13}$C-0.2% treatment. During the treatment period, there was no significant difference in the dry weight of shoots, root/shoot ratio and total biomass between different concentration treatments. The results showed that an elevated root-zone CO$_2$ concentration inhibited the accumulation of dry matter in roots with an increase in treatment time, but the effect on other types of biomass accumulation was insignificant.
Figure 5. High root-zone $^{13}$CO$_2$ affects the accumulation of biomass in roots and shoots of oriental melon. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.

2.6. Root-Zone $^{13}$CO$_2$ Concentration Affects the Total Carbon Content in Roots, Stems and Leaves of Oriental Melon

Carbon content can indicate the ability of plants to fix and store carbon. It can be seen from Figure 6 that, with an increase in treatment time, the carbon content in the roots and stems under the $^{13}$C-0.5%, $^{13}$C-1% and $^{13}$C-0.2% treatments increased. During the treatment, the carbon content under the $^{13}$C-0.2% treatment was significantly lower than those under $^{13}$C-0.5% and $^{13}$C-1% treatments in roots, and the $^{13}$C-1% treatment yielded significantly higher values than $^{13}$C-0.5%. The $^{13}$C-0.2% treatment yielded significantly higher values than $^{13}$C-0.5% and $^{13}$C-1% treatments in stems and leaves. The results showed that the higher the root-zone CO$_2$ concentration of oriental melon, the more carbon became fixed in the root system.

Figure 6. High root-zone $^{13}$CO$_2$ affects the total carbon content in roots, stems and leaves of oriental melon. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.
2.7. Root-Zone $^{13}$CO$_2$ Concentration Affects the Accumulation of Carbon in Roots, Stems and Leaves of Oriental Melon

The amount of carbon accumulation in the plant is the most intuitive indicator of carbon fixation. It can be seen from Figure 7 that the carbon accumulation in roots, stems and leaves increased with the increase in treatment time. The carbon accumulation under the $^{13}$C-0.2%, $^{13}$C-0.5% and $^{13}$C-1% treatments was in the order of leaf > stem > root. The carbon accumulation was affected by the carbon content and dry matter quality. The carbon content and dry matter accumulation of various organs at different concentrations increased with treatment time. Therefore, with the growth of oriental melon, the carbon accumulation of roots, stems and leaves treated with $^{13}$C-0.2%, $^{13}$C-0.5% and $^{13}$C-1% increased. The carbon accumulation in roots, stems and leaves under $^{13}$C-0.2% treatment was significantly higher than those of the $^{13}$C-0.5% and $^{13}$C-1% treatments, and $^{13}$C-0.5% treatment yielded significantly higher values than $^{13}$C-1%. In conclusion, high root-zone CO$_2$ inhibited the carbon accumulation of oriental melon roots, stems and leaves. In other words, high root-zone CO$_2$ inhibited the carbon fixation in oriental melon, thus affecting carbon assimilation; the higher the root-zone CO$_2$ concentration, the more significant the inhibitory effect.

![Figure 7](image_url)

Figure 7. High root-zone $^{13}$CO$_2$ affects carbon accumulation in roots, stems and leaves of oriental melon. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.

2.8. Root-Zone CO$_2$ Concentration Affects the Activities of Carbon Assimilation-Related Enzymes in Oriental Melon

Rubisco, RCA, TK, FBA, SBPase, FBPase and other enzymes in plants are mainly involved in the dark reaction of photosynthesis, and play a role in carbon assimilation. As shown in Figure 8, on the third day of treatment, the activity of Rubisco, FBA, FBPase and TK in the 0.5% and 1% root-zone CO$_2$ treatments was significantly lower than that in the 0.2% treatment, and while the activity of SBPase was significantly higher than that in the 0.2% treatment, the RCA activity in the 0.5% treatment was significantly higher than that in the 0.2% treatment. On the sixth day of treatment, the activity of Rubisco, RCA, FBA, SBPase and TK in the 0.5% and 1% treatments was significantly higher than that in the 0.2% treatment, and FBPase activity in the 0.5% treatment was significantly higher than that in 0.2%. After the ninth day of treatment, the activity of Rubisco, RCA, FBA, SBPase, FBPase and TK in the 0.5% and 1% treatments was significantly lower than that
in the 0.2% treatment. The results showed that a high root-zone CO\textsubscript{2} concentration could significantly affect the activity of carbon assimilation-related enzymes in oriental melon, and 0.5% and 1% root-zone CO\textsubscript{2} concentrations could significantly inhibit the activity of carbon assimilation-related enzymes after 9 days: the higher the CO\textsubscript{2} concentration, the more significant the inhibitory effect, thus affecting the carbon assimilation of oriental melon seedlings. This is also one of the reasons that high root-zone CO\textsubscript{2} treatment inhibited the photosynthesis of oriental melon seedlings.

![Graphs showing the activities of carbon assimilation-related enzymes](image)

**Figure 8.** High root-zone CO\textsubscript{2} affects the activities of carbon assimilation-related enzymes in oriental melon. Standard errors (SEs) of the means are represented by error bars. Different letters in the same row indicate significance at the 0.05 level among treatments.

### 2.9. Root-Zone CO\textsubscript{2} Concentration Affects the Expression of Carbon Assimilation-Related Enzyme Genes in Oriental Melon

Plant photosynthetic carbon assimilation is directly affected by the activities of various carbon assimilation-related enzymes. At the same time, the activities of carbon assimilation-related enzymes are also affected by the gene expressions of carbon assimilation-related enzymes in plants. Figure 9 shows the relative expressions of carbon assimilation-related enzyme genes in oriental melon seedlings under high root-zone CO\textsubscript{2} treatment. It can be seen from the figure that high root-zone CO\textsubscript{2} had a significant effect on the expressions of carbon assimilation-related enzyme genes in the leaves of oriental melon seedlings. The expression levels of CmRCA, which determines the carboxylation efficiency of Rubisco,
and CmSBPase, which regulates carbon influx, were significantly higher in the 0.5% and 1% treatments than in the 0.2% treatment at 3–6 days, and significantly lower in the 0.5% and 1% treatments than the 0.2% treatment after 9 days. This indicates that a high root-zone CO₂ concentration can promote CmRCA and CmSBPase expressions in the short term, and increase the syntheses of SBPase and RCA; the Rubisco carboxylation efficiency and carbon inflow also increased. Long-term high root-zone CO₂ concentration treatment can inhibit gene expression, negatively regulating the syntheses of SBPase and RCA. The expressions of CmRubisco, CmPRK and CmFBA, which regulate photosynthesis and CmFBPase, which affects carbohydrate accumulation and photosynthetic efficiency, were significantly lower in the 0.5% and 1% treatments on the third day than in the 0.2% treatment, significantly higher than the 0.2% treatment on the sixth day and significantly lower on the ninth day than in the 0.2% treatment. The expression of CmFBPase was only observed under the 0.5% treatment, which was significantly higher than 0.2% on the sixth day. The expression of CmTK, involved in photosynthetic carbon fixation, under the 0.5% and 1% treatments was significantly lower than that of the 0.2% treatment on the third day, significantly higher than that of the 0.2% treatment on the sixth day and significantly lower than that of the 0.2% treatment on the ninth to the twelfth day. The expression of CmPGK in the 0.5% and 1% treatments on the third day was significantly lower than that in the 0.2% treatment, and that in the 1% treatment on the sixth day was significantly higher than that in the 0.2% treatment; CmPGK was significantly lower in the 0.5% and 1% treatments than in the 0.2% treatment after 12 days, which indicated that long-term high root-zone CO₂ treatment could cause the expressions of CmRubisco, CmFBPase, CmFBA, CmPRK, CmTK and CmPGK to be down-regulated, thus inhibiting the syntheses of related enzymes and affecting the carbohydrate accumulation and photosynthetic rate. In conclusion, after 9 days of high root-zone CO₂ concentration treatment, the expressions of carbon assimilation-related enzyme genes in oriental melon were down-regulated, and the activities of carbon assimilation-related enzymes were inhibited, thus inhibiting carbon assimilation in oriental melon.

Figure 9. Cont.
with higher root-zone values than 0.5%. The study found that higher dissolved carbon can be released under a high concentration of CO2 and transported upward rapidly. Moreover, 13C could be detected in all parts after treatment, and δ13C increased with treatment time, but the detection time of each part was different. This may be because the upward transport of CO2 can only be completed after it is absorbed by the root system for a certain time; previous studies estimated that 65–99% 13C was released to the atmosphere in 9 h to 4 weeks [34]. In this test, at the same time and in the same part, the δ13C in the 0.5% and 1% root-zone 13CO2 treatments is significantly higher than that in the 0.2% treatment. 13C can be detected first in the 0.5% and 1% 13CO2 treatments, and the 1% treatment yields significantly higher values than 0.5%. The study found that higher dissolved carbon can be released under a high concentration of CO2 [35]. The assimilation of xylem-transported CO2 is affected by the CO2 concentration in the xylem: the higher the CO2 concentration, the greater the 13C enrichment and assimilation [36]. L2 treated with 0.5% and 1% root-zone 13CO2 showed 13C before 0.2% treatment, which may be due to more 13C being absorbed by roots treated with higher root-zone 13CO2 and the faster upward transport speed.

After 13CO2 labeling in the rhizosphere, 13C can be detected in all organs of oriental melon (Figure 2). This study showed that when the 0.2%, 0.5% and 1% root-zone 13CO2 labeling treatments reached 24 h, δ13C in roots increased, and roots could absorb CO2. The absorbed CO2 is transported upward to the stems and leaves as the substrate of photosynthesis, or is diffused directly from the leaves to the atmosphere, resulting in a reduction in 13C in the roots [22] and decreasing the δ13C value in the roots after 24–72 h. Long-term high root-zone CO2 treatment increases the concentration of CO2 in root cells [37], which may reduce the ability of roots to absorb 13CO2. Compared with 0.2% root-zone 13CO2 treat-
ment, $^{13}$C in the 0.5% and 1% treatments was absorbed by roots and transported upward, to a greater extent under the 1% treatment than the 0.5%. Therefore, the $^{13}$C content in roots decreased more significantly, and the amount of $^{13}$C transported to stems and leaves decreased. High root-zone CO$_2$ treatment will affect the transportation capacity of xylem to water and nutrient elements, slow down the transportation of $^{13}$C absorbed by roots to stems and leaves, release $^{13}$C via respiration in stems and leaves and increase the dry mass of stems and leaves; the result is that $\delta^{13}$C is diluted, leading to a decrease in $\delta^{13}$C in stems and leaves over 24–72 h. The increase in $\delta^{13}$C in stems and leaves in the 0.2% treatment may be due to the continuous upward transportation of $^{13}$C after being absorbed by the roots, and the upward transportation content is higher than its loss. During the labeling period of the 0.2% root-zone $^{13}$CO$_2$ treatment, the size of $\delta^{13}$C was in the order of root > stem > leaf, which was consistent with the results of previous studies [38]. The $\delta^{13}$C value under the 0.5% and 1% $^{13}$CO$_2$ treatments is in the order of stem > root > leaf, which may be due to the fact that the increase in root-zone $^{13}$CO$_2$ concentration specifically promotes root growth in the short term [39]. Oriental melon seedlings have a higher transpiration rate, which facilitates the upward transport of carbon dioxide, enhances the photosynthesis of stems and intercepts the carbon dioxide diffused into the atmosphere [20].

In the process of treatment, the distribution of $^{13}$C in roots, stems and leaves showed that the 0.5% and 1% root-zone $^{13}$CO$_2$ treatments were significantly more effective than the 0.2% treatment (Figure 3). With an increase in labeling time, the $\delta^{13}$C in stems and leaves under the 0.2% treatment increased, the $\delta^{13}$C in roots almost did not decrease within 24–72 h, and the dry mass in various organs increased. Therefore, the distribution of $^{13}$C in roots, stems and leaves increased. At 24–72 h, although the dry matter accumulation under the 0.5% and 1% treatments increased, the $\delta^{13}$C in each organ decreased, resulting in $^{13}$C distribution. Moreover, the 0.5% and 1% root-zone $^{13}$CO$_2$ treatments significantly increased the $^{13}$C distribution ratio in leaves compared to the 0.2% treatment (Figure 4), which may be due to the fact that the CO$_2$ content in the greenhouse could not completely fulfill the needs of oriental melon leaves for photosynthesis. The carbon that was absorbed in the rhizosphere was transported to the leaves as an alternate carbon source to participate in photosynthesis, or the high root-zone CO$_2$ inhibited the photosynthesis in oriental melon [40,41]. The proportion of photosynthetic carbon allocated to the lower part of the ground during plant growth is reduced [42], which may cause more carbon to be absorbed by the root system and transported to the leaves for photosynthesis under high CO$_2$ stress in the rhizosphere. Previous studies have shown that the distribution of $^{13}$C absorbed by plants is affected by many factors [43,44]. Both root-zone $^{13}$CO$_2$ concentration and treatment time will affect carbon distribution.

Carbon content can be used to indicate the carbon fixation capacity of plants. Plants mainly absorb and assimilate a large amount of CO$_2$ through photosynthesis. With an increase in treatment time, the carbon content in each organ increased. The carbon content of roots under the 0.5% and 1% treatments was significantly higher than that of the 0.2% treatment and lower than that of the 0.2% treatment in leaves. The reason for this may be that more carbon was absorbed by roots under the 0.5% and 1% root-zone CO$_2$ treatments, resulting in an increase in carbon content in roots; alternately, it may have been an initial stress response or an increase in root-zone temperature, which is conducive to the transfer of photosynthetic products from leaves to roots [45]. The research shows that high root-zone CO$_2$ treatment enhances root nitrogen metabolism, so it is necessary to provide a carbon source in the upper part and reduce the carbon content in leaves [46]. Therefore, the carbon content under the 0.5% and 1% root-zone CO$_2$ treatments is higher than that under the 0.2% treatment in roots and lower than that under the 0.2% treatment in leaves.

The carbon accumulation in plants reflects the material accumulation from photosynthesis by plants using various growth factors [47]. Plant dry weight and carbon content determine the amount of carbon accumulation. In addition, photosynthetic carbon tends to accumulate in roots, stems and leaves during vegetative growth [48]. There was greater carbon content in roots treated with the 0.5% and 1% root-zone CO$_2$ compared to those under
the 0.2% treatment, and the dry matter accumulation was lower than that under the 0.2% treatment; meanwhile, the carbon content and dry matter accumulation of stems and leaves were lower than those under the 0.2% treatment; thus, 0.2% root-zone CO$_2$ concentration treatment led to higher carbon accumulation in stems and leaves than for the 0.5% and 1% treatments. Although the carbon content of roots that were treated with 0.2% root-zone CO$_2$ was lower than that of the 0.5% and 1% treatments, the dry matter accumulation was higher than that of the 0.5% and 1% treatments; thus, the carbon accumulation of roots treated with the 0.2% treatment was higher than that of the 0.5% and 1% treatments (Figure 7). The results showed that carbon accumulation was inhibited under a root-zone CO$_2$ concentration greater than 0.5%, which inhibited carbon fixation and affected carbon assimilation. Studies have shown that with an increase in dissolved inorganic carbon in the rhizosphere of plants, biomass accumulation will increase, but the absorption of nutrients by plants may also change [49]. An increase in CO$_2$ concentration significantly improves plants’ carbon absorption capacity and promotes plants’ carbon accumulation [50–53]. However, the carbon accumulation under high root-zone CO$_2$ treatment was inhibited, which may have been due to the decline in plant photosynthetic capacity caused by high CO$_2$ enrichment in the rhizosphere, which is not conducive to carbon accumulation in plant organs.

3.2. Elevated Root-Zone CO$_2$ Affects Carbon Assimilation of Oriental Melon Seedlings

The Calvin cycle is the primary pathway of carbon assimilation in C$_3$ plants. Rubisco, RCA, TK, FBPase, SBPase, FBA and other enzymes are the key enzymes in the Calvin cycle. Rubisco can be used to fix CO$_2$ and determine the level of the net photosynthetic rate. It is the key enzyme in photosynthesis and the rate-limiting enzyme in CO$_2$ assimilation [54]. Rubisco has little effect on the photosynthetic rate and can promote and stabilize Rubisco enzyme activity [25]. SBPase can maintain the regeneration of RuBP and the flow of carbon in the Calvin cycle, which plays an important role in carbon assimilation [28]. FBA controls the photosynthetic rate. A slight decrease in TK activity will significantly decrease the plant photosynthetic rate [26]. FBPase is a regulatory enzyme in the Calvin cycle and plays an important role in photosynthetic product transport [27]. Rubisco, RCA, FBPase and thioredoxin (Trx) affect plant photosynthesis [55]. Current studies have shown that root-zone CO$_2$ enrichment can inhibit photosynthesis [56], but the internal mechanism of the effect of high root-zone CO$_2$ on carbon assimilation-related enzymes remains to be studied. It was found that photosynthetic carbon assimilation enzyme activity would affect its carbon assimilation [57]. The activities of enzymes related to carbon assimilation affect the photosynthetic carbon assimilation of plants. This study found that, from the ninth day after treatment, the activity of Rubisco, RCA, TK, FBPase, SBPase and FBA decreased significantly, indicating that long-term 0.5% and 1% high root-zone CO$_2$ treatments inhibited the activities of carbon assimilation-related enzymes, thus inhibiting photosynthesis in oriental melon seedlings. The gene expressions of carbon assimilation-related enzymes will affect enzyme activity, thus affecting the ability of plants to assimilate CO$_2$ and then regulate carbon assimilation. After 9 days of treatment, the expressions of \textit{Cm}RCA, \textit{Cm}SBPase, \textit{Cm}FBPase, \textit{Cm}FBA, \textit{Cm}PRK and \textit{Cm}Rubisco under the 0.5% and 1% treatments were significantly lower than those in the 0.2% treatment, and the expressions of \textit{Cm}TK and \textit{Cm}PGK in the 0.5% and 1% treatments were significantly lower than those under 0.2% after 9–12 days and 12 days, respectively. The results showed that long-term high root-zone CO$_2$ treatment decreased the expressions of essential enzyme genes in carbon assimilation, decreased the activities of carbon assimilation-related enzymes and inhibited photosynthetic carbon assimilation. Carbon assimilation is one of the main means of enrichment under the condition of elevated root-zone CO$_2$ [58]. The above results reveal the effect of high root-zone CO$_2$ on plant carbon assimilation from the perspective of carbon assimilation-related enzymes. In addition, the gene expressions of carbon assimilation-related enzymes are affected by high root-zone CO$_2$, which regulates internal enzyme activities through gene expression, and then regulates the photosynthetic carbon assimilation of plants.
4. Materials and Methods

4.1. Plant Materials and Growth Conditions

Oriental melon, of the ‘Yumeiren’ cultivar, was grown aeroponically in the greenhouse at Shenyang Agricultural University, Shenyang, Liaoning, China. The test was carried out when the oriental melon seedlings grew to three real-leaf stages. Yamazaki nutrient solution for oriental melon was supplied through a pump. The nutrient solution was replaced every 4 days. When the seedlings grew to five real-leaf stages, oriental melon seedlings with uniform size were selected for isotope labeling. During the labeling period, the temperature was 25–30 °C in the daytime, with adequate illumination, and 15–20 °C at night; the relative humidity of the air was 50–60%.

4.2. Isotopic 13CO2 Feeding Experiment

A 13C carbonic acid (Na213CO3) stable isotopic tracer experiment was performed (as shown in Figure 10). The 13CO2 stable isotope marking box was composed of glass. The marking box’s length, width and height were 50 cm, 50 cm and 25 cm, respectively. A cultivation hole with a diameter of 2 cm was drilled every 10 cm above the marking box, and 9 seedlings could be planted in each marking box. A 100 mL beaker (containing labeled Na213CO3) was fixed on the box’s inner wall. Na213CO3 (99 atom% 13C) was used for feeding treatment, and Na2CO3 was used as the control for unlabeled CO2 treatment. Six holes with a diameter of 1 cm were set 5 cm above the side of the marking box, with a hole spacing of 10 cm. A rubber tube was inserted into the middle of the liquid nutrient level and gas part to measure the 13CO2 (CO2) concentration in the marking box. The CO2 absorption device was connected through the rubber tube in hole 2. Before feeding, the gas in the feeding box was extracted through the rubber tube by the air pump, the CO2 component in the gas in the box was removed through the washing bottle containing NaOH solution, and then the other gases except CO2 were sent back to the feeding box through the rubber tube in hole 3. Hole 4 of the marking box was connected to the O2 increasing pump to maintain the O2 concentration of the root system in the marking box at a normal level. Hole 5 was connected to a syringe containing dilute sulfuric acid (2 mol L\(^{-1}\)). When feeding began, dilute sulfuric acid was injected into the beaker containing Na213CO3, and a particular concentration of 13CO2 (CO2) was produced after the reaction. A small fan was installed in hole 6 to ensure that the gas 13CO2 (CO2) concentration in the marking box was uniform. We sealed all interfaces of the marking box with sealant to keep the marking box closed during feeding. The outer layer of the marking box was covered with a black film during the treatment, and the plants were fixed on the cultivation hole with a rubber stopper to keep the root system within the marking box. We placed an appropriate amount of nutrient solution (pH 6.5–6.8) into the box to cause 1/3 of the root system of oriental melon to come into contact with the nutrient solution. The device obtained the national utility model patent (Patent No.: ZL 201920165969.2).

Figure 10. Isotope tracer processing system.
4.2.1. Root-Zone CO2 Concentration Treatment

At the beginning of feeding, we used a syringe to inject 50 mL dilute sulfuric acid (2 mol L\(^{-1}\)) into the beaker, which reacted with Na\(_2^{13}\)CO\(_3\)(Na\(_2^{12}\)CO\(_3\)) to produce \(^{13}\)CO\(_2\) (CO\(_2\)) gas. After injection, the rubber tube and orifice were sealed. In the process of feeding, we turned on the electric fan in the closed marking box in order to ensure that a uniform gas concentration in the box and consistent marking intensity were maintained for oriental melon seedling roots in the same marking box.

We implemented 0.2% (0.2% ± 0.0005%), 0.5% and 1% root-zone \(^{13}\)CO\(_2\) concentration treatments (0.2% is CK, conventional root-zone CO\(_2\) concentration measured in the early stage; 0.5% and 1% are high root-zone CO\(_2\) concentrations). The unlabeled 0.2%, 0.5% and 1% CO\(_2\) concentration treatments were used as the control \(^{13}\)C labeled treatments, which were named C-0.2%, C-0.5%, C-1%, \(^{13}\)C-0.2%, \(^{13}\)C-0.5% and \(^{13}\)C-1%, respectively. The sampling of each treatment was repeated three times.

4.2.2. Sampling Period and Method

In order to clarify the time limit of CO\(_2\) absorption by oriental melon roots and transportation to the aboveground part, samples were taken at 0, 0.5, 1.5 and 5 h after feeding. The plants were divided into three parts for sampling (Figure 11). The root was the first part (named L1); the first and second real-leaf and the first and second stems of the plant constituted the second part (named L2), and the other leaves and stems comprised the third part (named L3). Samples were washed in distilled water and dried with filter paper during sampling. The labeled samples were sterilized at 105 °C for 30 minutes, and dried at 75 °C over 72 h. The isotopic composition (δ\(^{13}\)C) of the sample was measured with an isotope ratio mass spectrometer that was connected to an elemental analyzer (Elementar vario PYRO cube-IsoPrime100, Hanau, Germany).

![Plant](image)

Figure 11. Sampling site of isotopic \(^{13}\)CO\(_2\) feeding plant.

The results of the \(^{13}\)CO\(_2\) stable isotope tracer experiment showed that oriental melon roots could absorb CO\(_2\) and transport it to the aboveground part. In order to study the distribution of carbon absorbed by roots in plants under different root-zone CO\(_2\) concentrations and treatment times, and considering that it was impossible to control the isotope labeling device for a long time, samples were taken at 0, 24 and 72 h after labeling, and the plant was divided into roots, stems and leaves. The treatment method was consistent with the above.
For the determination of carbon assimilation-related enzyme activity and related gene expression, the functional leaves (from the 3rd and 4th nodes above) of oriental melon seedlings that were subjected to 0.2%, 0.5% and 1% treatments were taken at 0, 3, 6, 9, 12 and 15 days after root-zone CO\textsubscript{2} ventilation treatment. For subsequent analyses, the samples were frozen in liquid nitrogen and stored in a refrigerator at \(-80^\circ\text{C}\).

4.3. Measurement Indicators and Methods

4.3.1. Determination of Carbon Content and \(\delta^{13}\)C Value

We weighed and placed the dried samples into the grinding prototype, and ground them through a 100-mesh sieve. Then, we placed 7~8 mg samples into a tin boat and wrapped them, and then determined the carbon content and \(\delta^{13}\)C value of the sample with an EA-IRMS (Elementar vario PYRO cube-IsoPrime100 Isotope Ratio Mass Spectrometer, Germany) (generally speaking, the plant carbon isotope abundance can be expressed by the \(\delta^{13}\)C value). The \(^{13}\)C distribution amount, \(^{13}\)C distribution proportion and carbon accumulation were calculated using the following formulas:

\[
\delta^{13}\text{C} (\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000
\]

where \(R_{\text{sample}}\) is the \(^{13}\)C/\(^{12}\)C atomic ratio of the sample, and \(R_{\text{standard}}\) is the \(^{13}\)C/\(^{12}\)C atomic ratio of the standard, which is 0.011802.

\(^{13}\)C distribution in each organ (mg):
\[
{^{13}\text{C}}_1 = C_1 \frac{(F_i - F_{il})}{100} \times 1000
\]

where \(C_1\) is the carbon accumulation of each component; \(F_i\) is the \(^{13}\)C abundance of the marker component; \(F_{il}\) is the \(^{13}\)C abundance of the unmarked component.

Proportion of \(^{13}\)C distribution in each organ (%): \(\frac{{^{13}\text{C}}_1}{{^{13}\text{C}}_{\text{distribution}}} \times 100\)

where \(^{13}\text{C}_{\text{distribution}}\) is the sum of \(^{13}\)C distribution of roots, stems and leaves.

Carbon accumulation of each part (mg) = \(C \times 1000 \times \text{DW} \text{ (g)}\)

where \(C\) is the carbon content of each part of the root, stem and leaf, and DW is the dry weight of each part.

4.3.2. Determination of Carbon Assimilation-Related Enzyme Activity and Gene Expression

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), Rubisco activating enzyme (RCA), fructose-1,6-diphosphate esterase (FBPase), fructose 1,6-bisphosphate aldolase (FBA), sedoheptulose-1,7-bisphosphatase (SBPase) and transketolase (TK) were determined using a kit (Jiangsu Boshen Biotechnology Co., Ltd., Jiangsu, China). Eight genes involved in photosynthetic carbon assimilation were analyzed using qRT-PCR with gene-specific primers. RNA extraction from leaves was carried out according to the instructions for the ultrapure RNA Kit (Beijing Kangwei century biology Co., Ltd., Beijing, China). The synthesis of cDNA was carried out according to the instructions for the reverse tran-scription Kit (Monad Biotechnology Co., Ltd., Suzhou, China). Fluorescence quantitative reaction was carried out on a Jena quantitative PCR instrument. The expression amount of each gene was calculated by the fluorescence quantitative kit operation method (DRR04A, TANGEN). The PCR reaction procedure used was as follows: 95 °C 30 s; 95 °C 5 s; 60 °C 34 s; 60 °C 15 s, 45 cycles. The relative gene expression was calculated via the \(2^{-\Delta\Delta\text{Ct}}\) method, the primer sequences are shown in Table 1 and each sample measurement was repeated 3 times.
### Table 1. Primer list for real-time quantitative PCR.

| Gene       | Primers Sequences 5′-3′ Accession Number |
|------------|------------------------------------------|
| Actin      | (F)AAGGCAAACAGGGAGAAGATGA (R)AGCAAGGTCGAGACGTAGGATA MELO3C012252.2 |
| CmRubisco | (F)TCACGGTAACAGAAATCCACTG (R)TATGTCCTGCTGCTTCACGGTAC MELO3C008231.2 |
| CmRCA      | (F)AAGGTGCTCGTTTTGCTAAGTG (R)TGTCCTGTCAATGGAATGGTCT MELO3C005333.2 |
| CmFBA      | (F)TCCTCGTCTCCCTCCTCCA (R)GCCATCACAGCAACTTTTCCA MELO3C018610.2 |
| CmFBPase   | (F)GGTTCCAGGCTACGAAACTTTCCA (R)AAATCCCAGATAATCAATGATGCT MELO3C025149.2 |
| CmSBPase   | (F)CTTGGATAGAGCATACCCATACG (R)CAACTCCCCTGGATAACTACAC MELO3C009351.2 |
| CmTK       | (F)ACAGTCTCTACAGCACAAGTCCCT (R)AAAGTCTTTTCCCAACCCCT MELO3C013811.2 |
| CmPRK      | (F)CTTTGGTATAGACGATAACACCCCATACG (R)CAACTCCTGGATAACTACAC MELO3C009351.2 |

F: Forward. R: Reverse.

#### 4.4. Statistical Analysis

Data were presented as means ± standard errors (SEs) and analyzed using variance analysis (ANOVA) in SPSS 22.0 (IBM, Armonk, NY, USA). Duncan’s multiple range tests were used to perform significance analysis under conditions of $p < 0.05$. Excel 2010 software was used to perform the data collation and mapping.

#### 5. Conclusions

In this study, the isotope tracer test and high root-zone CO$_2$ concentration test confirmed that oriental melon roots could absorb CO$_2$, and that the root-zone CO$_2$ concentration affected plant root carbon absorption and the transportation rate. The carbon absorption and distribution in various organs in oriental melon seedlings were significantly affected by high root-zone CO$_2$ concentration. The higher the root-zone CO$_2$ concentration, the more carbon was absorbed by the root, the faster the upward transportation speed was, the greater the values in the root, stem and leaf and the higher the proportion that was distributed in the leaf. High root-zone CO$_2$ down-regulated the gene expressions of carbon assimilation-related enzymes to affect the activities of carbon assimilation-related enzymes and inhibit the carbon assimilation of oriental melon seedlings.

### Author Contributions:

Conceptualization, X.H. and Y.J.; Methodology, X.H. and Y.J.; Validation, Y.L.; Formal analysis, X.H. and C.X.; Investigation, X.H. and L.G.; Data curation, X.H., M.L. and Y.L.; Writing—original draft preparation, X.H., Y.J. and Y.L.; Writing—review and editing, H.Q., C.X. and Y.L.; Supervision, H.Q.; Funding acquisition, Y.L. All authors have read and agreed to the published version of the manuscript.

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### Conflicts of Interest:

The authors declare no conflict of interest.

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