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How Elevated CO_2 Shifts Root Water Uptake Pattern of Crop? Lessons from Climate Chamber Experiments and Isotopic Tracing Technique

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Abstract: Root water uptake plays an important role in water transport and carbon cycle among Groundwater–Soil–Plant–Atmosphere–Continuum. The acclimation of crops under elevated carbon dioxide concentrations (eCO_2) depends greatly on their capability to exploit soil water resources. Quantifying root water uptake and its relationship with crop growth under eCO_2 remains challenging. This study observed maize growth subjected to current CO_2 (400 ppm) and eCO_2 (700 ppm) treatments via a device combined with a climate chamber and weighing lysimeters. Root water uptake patterns were determined based on the isotopic tracing technique. The main water uptake depth shifted from 0−20 cm under current treatment to 20−40 cm under eCO_2 at the seedling growth stage. Maize took up 22.7% and 15.4% more soil water from a main uptake depth of 40−80 cm at jointing and tasseling stages in response to eCO_2, respectively. More soil water (8.0%) was absorbed from the 80−140 cm layer at the filling stage under eCO_2. Soil water contributions at the main uptake depth during seedling stage were negatively associated with leaf transpiration rate (T_r), net photosynthetic rate (P_n), and leaf area index (LAI) under both treatments, whereas significant positive correlations in the 40−80 cm layer under current treatment shifted to the 80−140 cm layer by eCO_2. Deep soil water benefited to improve T_r, P_n and LAI under both treatments. No significant correlation between soil water contributions in each layer and leaf water use efficiency was induced by eCO_2. This study enhanced our knowledge of crop water use acclimation to future eCO_2 and provides insights into agricultural water management.

Keywords: elevated CO_2; root water uptake; stable isotopes; maize

1. Introduction

Global food security is undergoing an increasing crisis, threatened by climate change. Elevated atmospheric carbon dioxide concentrations (eCO_2) have great impacts on water use, crop growth and yield [1–3]. Root water uptake is critical in water and carbon cycles in Groundwater–Soil–Plant–Atmosphere–Continuum (GSPAC) by influencing canopy transpiration and growth of crops [1,4]. Understanding the depth distribution and magnitude of root water uptake is important for planning irrigation and developing crop acclimation strategies to climate change [1,5]. The global CO_2 concentration is predicted to reach 700 ppm by the end of the 21st century [6]. Maize is the most important food crop and is widely cultivated around the globe. Global demand for maize production is projected to double by 2050 facing a notable increase in CO_2 [7,8]. Determining the root water uptake patterns and their relationships with the growth of maize under eCO_2 is crucial, in particular for agricultural water management in response to future climate change [1,9,10].

Numerous experiments and model predictions have concentrated on the effects of eCO_2 on maize growth, yield, and water use efficiency [8,11–14]. Exposure of maize to eCO_2 has several physiological effects. The main physiological effect of eCO_2 is the significant decrease in plant transpiration (11%–22%) with improving water use efficiency [12,13,15,16]. However, there are many controversies over the responses of leaf photosynthesis to eCO_2. Maize, as a C4 crop, is usually saturated at current CO_2 and does not show significant structural (leaf area) or photosynthetic acclimation to eCO_2 [15,17]. Nevertheless, a few controlled experimental studies showed an improvement of photosynthesis under sufficient water supply [18–20], while others argued that the growth of maize benefitted from the increasing CO_2 only under drought stress conditions [8,21,22]. These various feedbacks of plant ecophysiological indices will lead to a considerable discrepancy in the response of water utilization and yield of maize to eCO_2.

Maize root water uptake has been widely investigated using isotope tracing techniques in recent years [4,23–26]. The stable isotopes of hydrogen and oxygen (^2H and ^{18}O) are effective tools to quantify the proportional contribution of water sources to plant water uptake [10,27–30]. Most of the previous studies have determined the main root water uptake depth of maize and its seasonal variability among various irrigation and fertilization strategies. Maize across the globe was identified to primarily absorb shallow soil water (mostly at the upper 20 cm depth), particularly during the vegetative and mature stages [4,31]. Soil water in deep layers (even more than 120 cm) could be taken up by maize during the silking and milking stages [26]. The contributions of soil water sources were found to be positively correlated with the root length or dry root weight density [24,26], and they were negatively related to the soil water content for summer maize [26]. Nevertheless, to what extent soil water contributions at different depths will be altered through crop growth variations by eCO_2 is poorly known. To our knowledge, no previous study has investigated the seasonal variation in root water uptake pattern and its correlation with growth of maize under eCO_2.

In this study, maize growth under the current and eCO_2 treatments was monitored in an indoor device which was combined with a climate chamber, weighing lysimeters and groundwater supply systems. The ^2H and ^{18}O isotopes were used to determine the root water uptake patterns. The objectives of this study were: (1) to quantify the seasonal variations in the contributions of soil water sources to plant water uptake under current and eCO_2 treatments, (2) to compare the correlations between soil water contributions in different layers and plant ecophysiological indices at current and eCO_2 treatments, and (3) to investigate the impacts of eCO_2 on water use strategies.

2. Materials and Methods

2.1. Description of the Experiment

A coupled device with climate chamber and weighing lysimeters was used to conduct the experiments of maize in the Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China. The coupled device was composed of one climate chamber (7 m long, 5 m wide and 4.5 m high), two weighting lysimeters (3 m long, 2 m wide and 3 m deep), and a groundwater supply system (Figure 1). The climate chamber had a CO_2 gas storage cylinder connecting with a sensor transmitter and a flow velocity meter to control the CO_2 concentration. Two centrifugal humidifiers and an air conditioning system were utilized to control the humidity and air temperature, respectively. The air in the climate chamber was circulated using four fans. There were twenty-four sodium lamps and metal halide lamps to provide light source for crop growth in climate chamber.

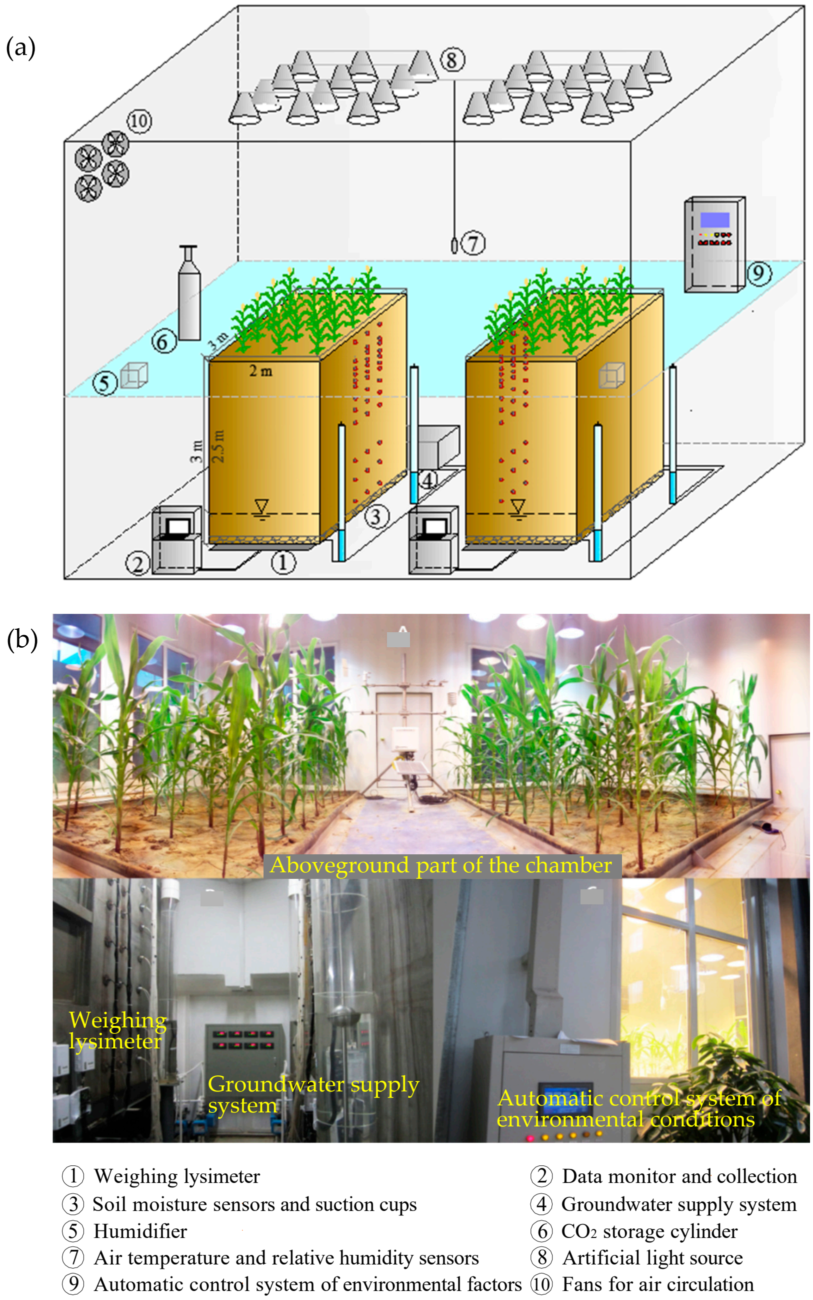

Figure 1. (**a**) Schematic diagram and (**b**) photographs of the coupled device with climate chamber and weighing lysimeters.

The two lysimeters were designed as replicates for experiments, which were filled with alluvial soil from farmlands in Beijing. The soil texture was sandy loam, consisting of 60.21% sand, 39.09% silt and 0.70% clay. It had a field capacity of 0.25 cm^3 cm^{-3} and a saturated water content of 0.48 cm^3 cm^{-3}. Seven 5TE sensors (Decagon Devices, Pullman, WA, USA) were installed at depths of 10, 30, 40, 80, 100, 120 and 170 cm below the soil surface in each lysimeter to monitor the soil water content (θ). Thirteen porous ceramic suction cups (DLS-II, IGSNRR, Beijing, China) were installed at depths of 10, 30, 40, 50, 60, 70, 80, 100, 120, 140, 170, 200, and 230 cm in each lysimeter to extract soil water for isotope analysis. A Mariotte bottle connected with a water storage tank was used for controlling the water table in each lysimeter.

Maize (cultivar ZhengDan 958) was sown with an intrarow spacing of 63 cm and interrow spacing of 37 cm in each lysimeter. It was treated at two different CO_2 concentrations in this study (Figure 1).

One treatment represents current CO_2 condition (400 ppm) conducted between 17 May 2014 to 19 September 2014, and the other treatment represents eCO_2 condition (700 ppm) predicted for the end of this century which was conducted from 26 October 2015 to 28 February 2016. The daily variations in the CO_2 concentrations during the growing season of maize under current and eCO_2 treatments are indicated in Figure 2. The growth periods for each treatment are shown in Table 1. Amount of irrigation and fertilization was same between the current and eCO_2 treatments (Table 1). The air temperature and relative humidity for the two treatments were mainly controlled to match those representing the normal climate conditions in the summer maize season, determined from the Beijing metrological station (116°28′ E, 39°48′ N) (Figure 2). The light intensity was set to 150 $W \cdot m^{-2}$ above the canopy from 6 a.m. to 6 p.m. and the water table depth kept at 2.5 m for both treatments.

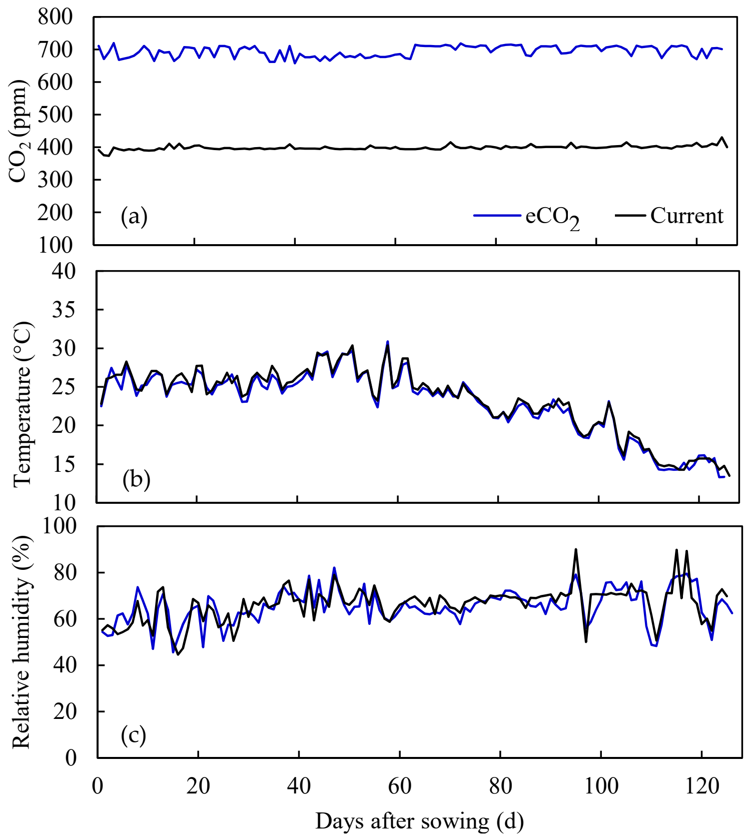

Figure 2. Daily variations in (**a**) carbon dioxide (CO_2) concentrations; (**b**) temperature and (**c**) relative humidity under current and elevated CO_2 (eCO_2) treatments.

Table 1. The growth stages and application of irrigation and fertilizer for maize under current and elevated CO_2 (eCO_2) treatments.

Stage	Days after Sowing		Amount of Irrigation (mm)	Fertilizer	
	Current	eCO_2		Type	Amount (kg ha^{-1})
Seedling	0–32	0–33	20	Urea	300
Jointing	33–64	34–66	60	None	None
Tasseling	65–79	67–82	60	Urea	300
Filling	80–103	83–105	None	None	None
Maturity	104–125	106–126	60	None	None

2.2. *Water Sampling and Isotope Analysis*

Samples of irrigation, soil water, and stem water were collected in two lysimeters as two replicates to analyze the differences in seasonal isotopic compositions (δ^2H and $\delta^{18}O$) between the current and

eCO_2 treatments. Irrigation water was sampled via a 50 ml polyethylene bottle and then sealed with parafilm. Soil water at depths of 10, 30, 40, 50, 60, 70, 80, 100, 120, 140, 170, 200, and 230 cm were collected using the porous ceramic suction cups installed in the weighing lysimeters (Figure 1). There were one or two sampling campaigns of soil water within the seedling (10 d and 31 d), jointing (43 d and 51 d), tasseling (72 d), and filling (94 d) growth stages under each treatment.

On the same dates for soil water collection, the stems between the first node and the soil surface of one representative plant in each lysimeter were sampled for isotopic analysis. They were cut into several 2 to 3 cm long pieces and all the epidermises were removed. All the samples of stems were then put into 4 ml glass bottles and kept at −15 °C and −20 °C via a refrigerator before isotope measurements. Stem water was extracted using the cryogenic vacuum distillation system (LI-2000, LICA, Beijing, China).

The isotopic compositions in different water samples were measured by the Los Gatos Research (LGR) liquid water isotope analyzer (DLT-100, Mountain View, CA, USA). The measured δ^2H and δ^{18}O values were calibrated using the Vienna Standard Mean Ocean Water (VSMOW) international standards. The precision was ±1‰ for δ^2H and ±0.1‰ for δ^{18}O measurements. Deuterium excess (d-excess) reflects the fractionation or isotopic enrichment of individual samples and is described as d-excess = δ^2H − 8.0 × δ^{18}O [32].

2.3. Measurements of Plant Ecophysiological Indices

Plant transpiration is the main force for root water uptake [10], and its fraction in evapotranspiration is positively correlated with the leaf area index (*LAI*) [33]. Carbon allocation to root growth may vary with any change in leaf photosynthesis and affect the water use strategies by roots [1]. Therefore, four typical growth indicators including transpiration rate (T_r), net photosynthetic rate (P_n), water use efficiency at leaf scale (WUE_L), and *LAI* were selected to explore their correlations with plant water uptake patterns in this study. Six representative plants were selected in each plot to measure the leaf growth characteristics of maize. These measurements were generally conducted on the sampling dates of soil water. The P_n and T_r were measured on the second leaf of the representative plants by an LI-6400 photosynthesis system (LI-COR Inc., USA) with a leaf room temperature of 28 °C and flow rate of 500 µmol s^{-1} [34]. The WUE_L was calculated as the P_n divided by the T_r. The measurements of maximum width and length of all leaves were conducted via a steel tape to calculate the leaf area for each plant. The *LAI* was estimated as the division of the total leaf area and related ground surface area per plant.

2.4. Quantification of Water Source Contributions to Maize

The MixSIAR Bayesian mixing model has the advantage of accounting for uncertainties in estimation of source contributions and providing an optimal solution rather than a range of feasible solutions [35]. It was particularly efficient in the case of over three potential water sources for plant water uptake [36]. In this study, dual stable isotopes together with the MixSIAR model (v2.1.3) were used to determine the seasonal variations in the main water uptake depth and quantify the contributions of soil water at different depths to maize water uptake. Soil water at different depths was considered as the primary water sources for maize, since soil water was a mixture of irrigation water, old soil water, and upward fluxes of groundwater in our experiments. The soil water sources were divided into five layers including 0−20, 20−40, 40−80, 80−140, and 140−250 cm, in terms of seasonal variations in the δ^2H and δ^{18}O isotopes. The δ^2H and δ^{18}O values of soil water averaged from those at all sampling depths within each layer were input as raw source data into the MixSIAR model. The isotopic compositions of stem water on the same dates were input as mixture data. For convergence, the run length of the Markov chain Monte Carlo (MCMC) parameter was specified as "very long". Both the process error and residual error were used to evaluate the model erroneous. The median (50% quantiles) proportions referred to the contributions of soil water in each layer to stem water in this study.

2.5. Statistical Analysis

Differences of the seasonal variations in the isotopic compositions of water sources and growth indicators were compared between the current and eCO_2 treatments via analysis of variance (ANOVA) with LSD (least significance difference). The correlations between the proportions of soil water contribution in different layers and plant ecophysiological indices under two treatments were estimated. All statistical analyses were conducted using the SPSS (version 22.0) software package (IBM Corp., Chicago, Illinois, USA). The level of statistical significance was determined as $p < 0.05$.

3. Results

3.1. Water Stable Isotopes under Current CO_2 and ECO_2 Treatments

For the current treatment, soil water isotopic values (δ^2H and $\delta^{18}O$) exhibited greater seasonal variation in the 0−80 cm layer than those in other soil layers (Figure 3). The isotopic compositions of soil water especially at the upper 20 cm layer were significantly more enriched at the tasseling and filling growth stages compared to the seedling and jointing stages ($p < 0.05$). The soil water isotopes within the 80−140 cm layer remained stable with depleted values over the entire observation period. Notably enriched soil water isotopes in the deep layer (140−250 cm) occurred at the filling stage for current treatment. Significant differences in the soil water isotopes between the current and eCO_2 treatments were characterized by enriched values in the 0−80 cm soil layer at the seedling and jointing stages ($p < 0.05$) (Figure 3). The more negative d-excess also indicated that the 0−80 cm soil layer was exposed to increased evaporation under eCO_2 compared to the current treatment at the seedling and jointing stages. The lowest d-excess in the 0−20 cm layer suggested the highest impact of evaporation near the soil surface under eCO_2 (Figure 3). It was found that eCO_2 resulted in larger variance in the soil water isotopic values within the 80−140 cm layer, but an inverse change appeared in the 140−250 cm layer.

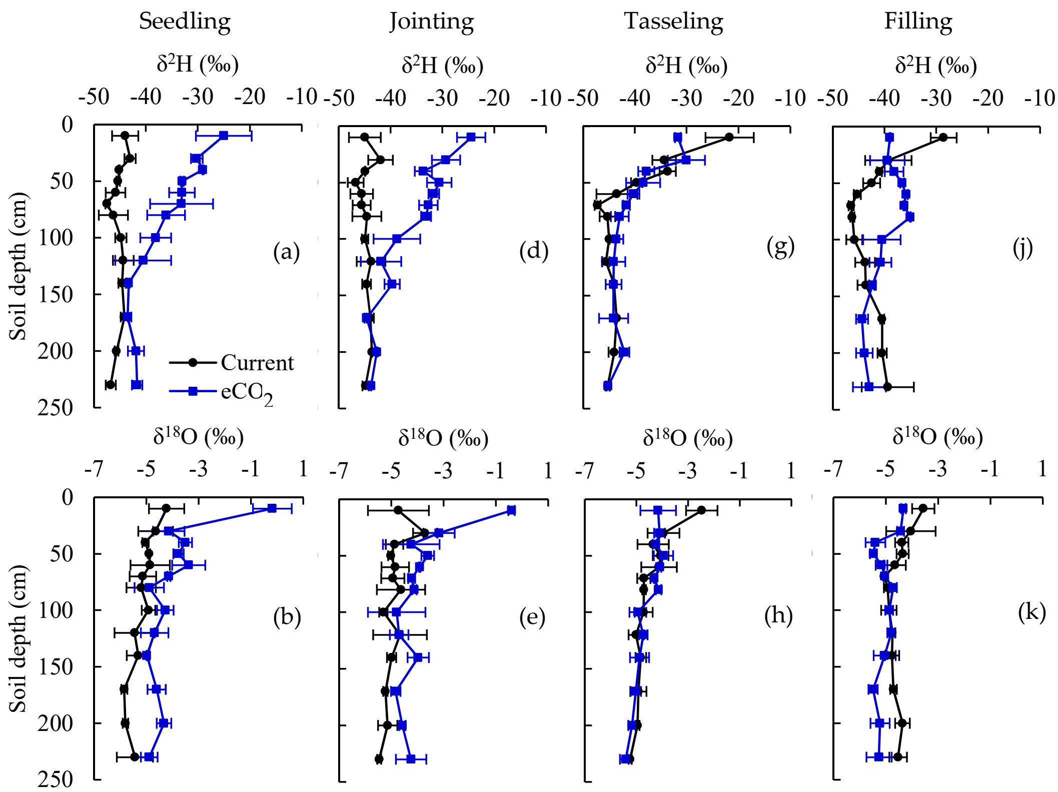

Figure 3. *Cont.*

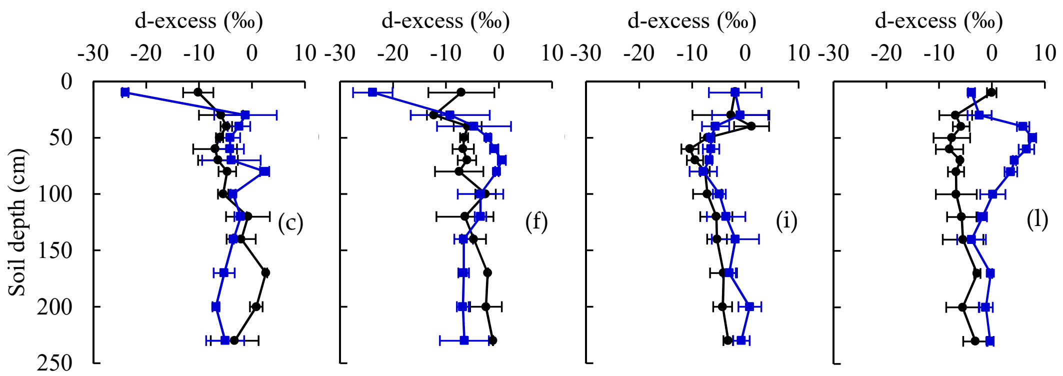

Figure 3. Depth distribution of δ^2H, δ^{18}O, and deuterium excess (d-excess) at seedling (**a**–**c**), jointing (**d**–**f**), tasseling (**g**–**i**), and filling (**j**–**l**) growth stages of maize under current and eCO_2 treatments, respectively.

All soil water isotopes under the current treatment were more enriched than irrigation water, and the soil water line was fitted as $\delta^2H = 5.80\ \delta^{18}O{-}15.51$ ($R^2 = 0.60$, $p < 0.001$) (Figure 4a). Soil water isotopes endured greater evaporation fractionation under eCO_2, indicated by a lower slope in the soil water line ($\delta^2H = 3.42\ \delta^{18}O23.22$, $R^2 = 0.54$, $p < 0.001$) (Figure 4b). Stable isotopes of stem water mainly fell on the soil water lines under both the current and elevated CO_2 treatments. Most stem water isotopes exhibited similar values to those of the soil water in the 40–80 cm layer over the observed growing period, except in the surface layer (0–20 cm) at the seedling stage for the current treatment (Figure 4a). Maize under eCO_2 had more positive δ^2H and δ^{18}O values in stem water at the seedling and jointing stages. They matched well with those enriched isotopic values in soil water, especially in the 20–40 cm and 40–80 cm layers, suggesting a higher reliance on water sources from these two layers for maize under eCO_2.

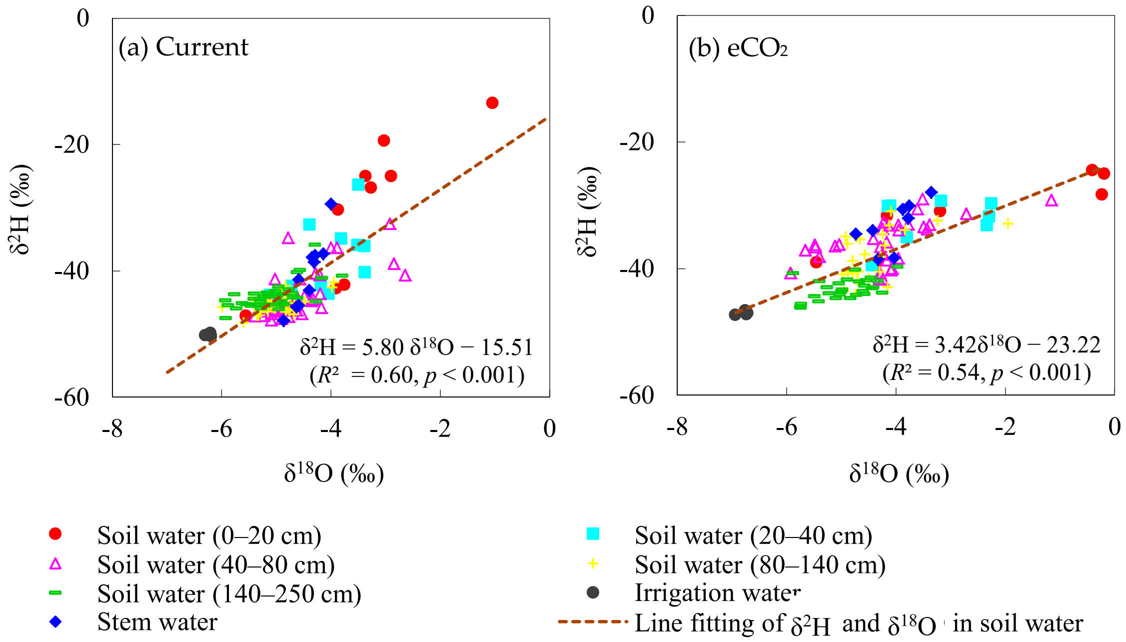

Figure 4. Isotopic values of stem water, irrigation water, and soil water in different layers under (**a**) current and (**b**) eCO_2 treatments, respectively.

3.2. Plant Ecophysiological Indices under Current CO_2 and eCO_2 Treatments

There were large differences of photosynthesis parameters of maize leaves between eCO_2 and current treatments (Figure 5a,b). The mean value of P_n was 7.2 (±0.2), 7.8 (±0.2), 9.4 (±0.1), and 10.6 (±0.5) umol m^{-2} s^{-1} at the seedling, jointing, tasseling, and filling growth stages for the current treatment, respectively (Figure 5b). Although P_n had an increasing trend from the seedling to filling stage under eCO_2, it was on average 10.2% lower than that under the current treatment (Figure 5b). The mean value of T_r increased rapidly from the seedling (0.6 mmol m^{-2} s^{-1}) to filling (1.7 mmol m^{-2} s^{-1}) stage compared to P_n under the current treatment (Figure 5a and b). However, eCO_2 resulted in a significantly greater reduction in T_r (with a mean of 28.1%, $p < 0.05$), especially at the filling stage with respect to changes in P_n. Maize leaves developed rapidly at the jointing stage and then *LAI* reached the maximum value at the tasseling stage for both treatments (mean of 3.1) (Figure 5c). However, eCO_2 decreased *LAI* slightly by 4.4% during the first three growth stages but increased with a mean of 4.4% at the filling stage compared to the current treatment (Figure 5c). The differences of *LAI* under current and eCO_2 treatments during the entire growth period were not significant ($p > 0.05$). It was evident that the mean of WUE_L decreased greatly from 9.3 to 6.4 umol $mmol^{-1}$ during the observed growth period under the current treatment (Figure 5d). Higher WUE_L (with a mean of 30.5%) was induced by eCO_2, particularly at the tasseling and filling stages ($p < 0.05$).

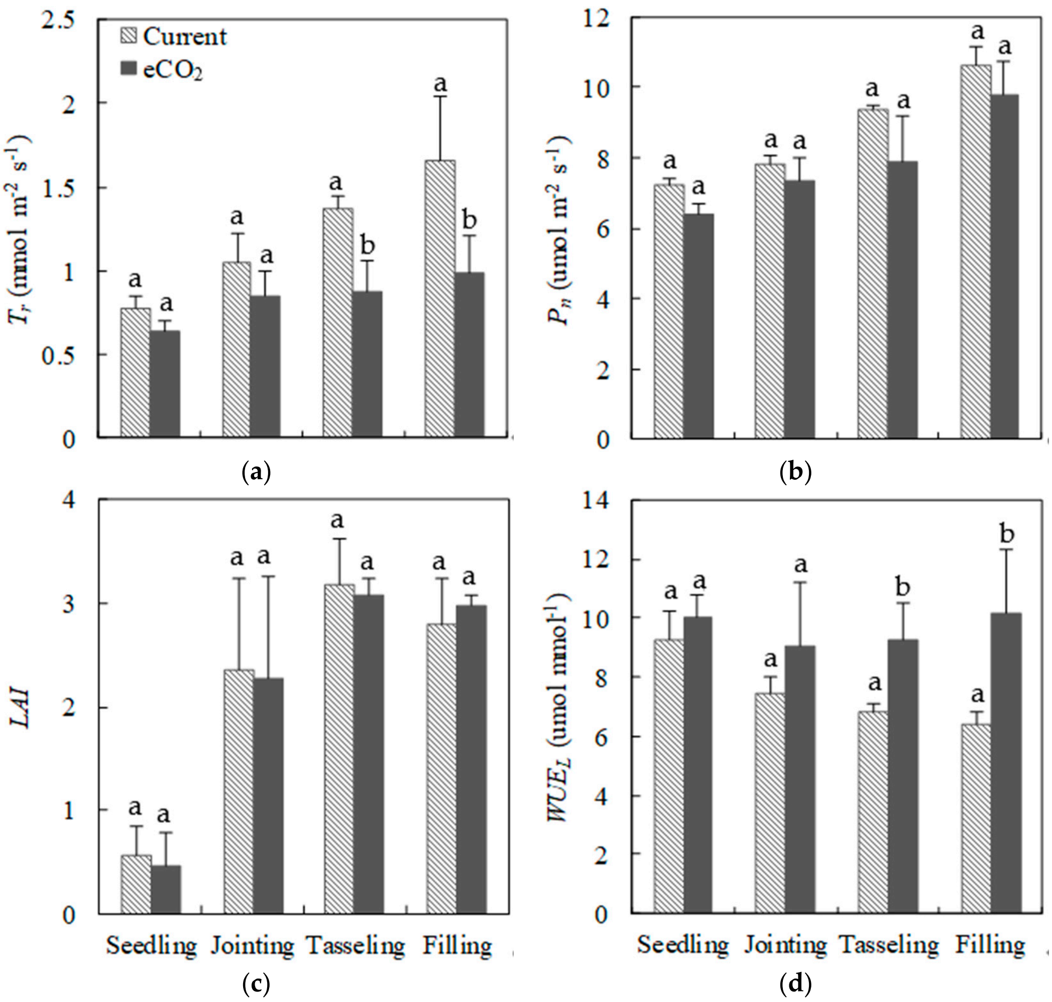

Figure 5. Seasonal variations in the (**a**) transpiration rate (T_r), (**b**) net photosynthetic rate (P_n), (**c**) leaf area index (*LAI*), and (**d**) leaf water use efficiency (WUE_L) of maize at different growth stages under current and eCO_2 treatments, respectively.

3.3. Root Water Uptake Patterns under Current CO_2 and eCO_2 Treatments

The main water source for maize was soil water in the 0–20 cm layer at the seedling stage and in the 40–80 cm layer at other growth stages under the current treatment (Figure 6a). The soil water in the 0–20 cm layer contributed to a maximum of 44.0% of maize water uptake at the seedling stage. The contribution of soil water in the 20–40 layer remained at low values (ranging between 8.9 and 19.4%) at the seedling to tasseling stages, while this contribution increased to 24.1% (±16.5%) at the filling stage. Maize greatly relied on the water source from the 40–80 cm soil layer, starting from the jointing stage, taking up water with a mean of 29.2% (±3.8%) from this layer (Figure 6a). There was a stable contribution of soil water in the 80–140 cm layer (17.6% ± 2.4%) under the current treatment. Soil water in the 140–250 cm layer, providing 22.0% (±7.9%) water to plant water take, played an important role in water utilization by maize, especially at the tasseling and filling stages (Figure 6a).

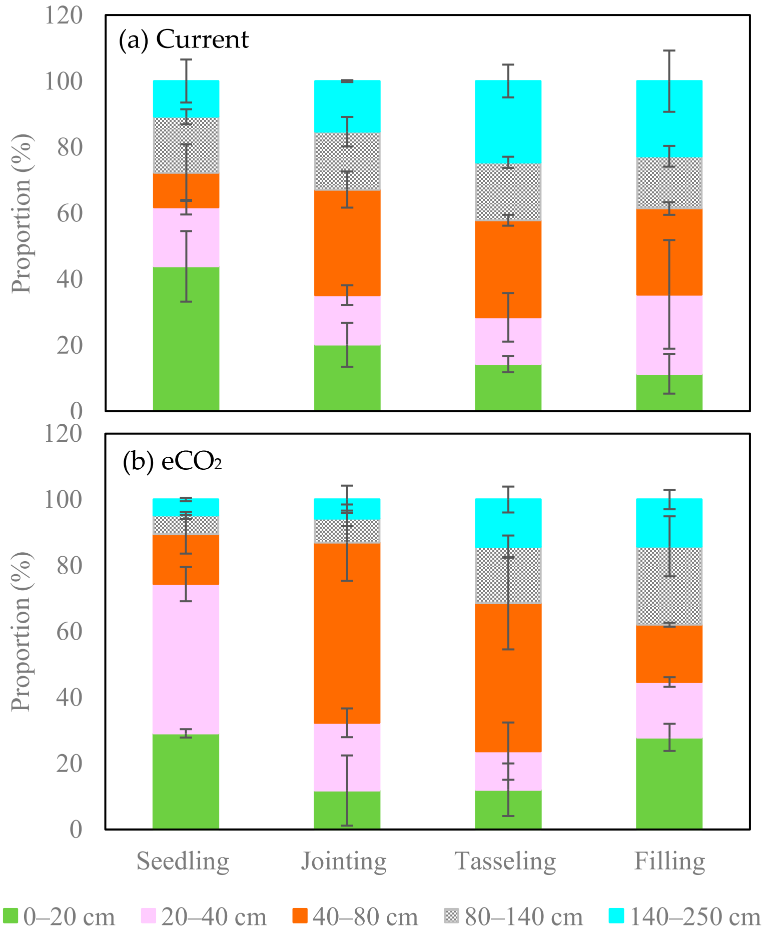

Figure 6. Proportions of soil water contribution in each layer at different growth stages under (**a**) current and (**b**) eCO_2 treatments, respectively.

Compared to the current treatment, the main water uptake depth shifted from 0–20 cm to 20–40 cm at the seedling stage under eCO_2 treatment (Figure 6b). The contribution of soil water in the 20–40 layer at this stage was 27.4% higher than that for maize exposed to the current treatment (Figure 6b). The main water uptake depth at the jointing and tasseling stages under eCO_2 was the 40–80 cm layer, which was same as under current treatment. Nevertheless, the soil water contribution in 40–80 cm layer was 22.7% and 15.4% higher at the jointing and tasseling stages under eCO_2, respectively (Figure 6b). More soil water was absorbed from the 0–20 cm (16.6%) and 80–140 cm (8.0%) layers at the filling stage for maize under eCO_2 treatment. There was an average 8.6% reduction in the contribution of water source from the deep layer (140–250 cm) to maize water uptake over the entire growth period under eCO_2 treatment with respect to current treatment (Figure 6b).

3.4. Correlations between Root Water Uptake Patterns and Crop Growth under Current and eCO_2 Treatments

For maize under the current treatment, significant negative correlations were found between the proportions of soil water contribution in the 0−20 cm layer and T_r, P_n and *LAI* ($p < 0.01$), as shown in Table 2. The T_r, P_n and *LAI* showed significant positive correlations ($p < 0.05$) with the water contributions in both the 40−80 cm and 140−250 cm soil layers under the current treatment, suggesting greater reliance of leaf development on soil water in these two layers. No correlations were found between the soil water contributions in the 20−40 cm and 80−140 cm layers and T_r, P_n as well as *LAI* under the current treatment. The WUE_L was positively related to the soil water contributions in the 0−20 cm layer ($p < 0.05$), indicating that maize took up more surface soil water for P_n rather than T_r. On the contrary, there were negative effects of soil water contribution in the 140−250 cm layer on WUE_L.

Table 2. Correlation analysis between proportions of soil water contribution in different layers and ecophysiological indices of maize during the seedling to filling growth period under current and eCO_2 treatments.

Proportions in Each Layer	T_r		P_n		*LAI*		WUE_L	
	Current	**eCO_2**	**Current**	**eCO_2**	**Current**	**eCO_2**	**Current**	**eCO_2**
0–20 cm	**−0.84 ****	0.00	**−0.92 ****	0.00	**−0.91 ****	−0.14	**0.64 ***	0.00
20–40 cm	0.00	**−0.54 ***	0.01	**−0.58 ***	−0.01	**−0.88 ****	0.00	−0.05
40–80 cm	**0.53 ***	0.00	**0.61 ***	0.00	**0.83 ***	0.09	−0.38	0.01
80–140 cm	−0.06	**0.67 ***	−0.01	**0.67 ***	0.00	**0.58 ***	0.13	0.01
140–250 cm	**0.69 ***	**0.82 ****	**0.60 ***	**0.72 ****	**0.60 ***	**0.60 ***	**−0.73 ****	−0.03

Note: * Significant level $p < 0.05$, ** Significant level $p < 0.01$. Bold front represents the significant correlation ($p < 0.05$).

In comparison with the current treatment, eCO_2 modified the correlations between T_r, P_n and *LAI* of maize and soil water contributions at all layers except the deep layer of 140−250 cm (Table 2). There was no correlation between the soil water contributions in the 0−20 cm and 40−80 cm layers ($p > 0.05$) and T_r, P_n and *LAI* under eCO_2 treatment, compared to those significant correlations under the current treatment. However, the T_r, P_n and *LAI* were significantly correlated with soil water contributions in the 20−40 cm (negative) and 80−140 cm (positive) layers ($p < 0.05$) under eCO_2 treatment, in comparison with a nonsignificant correlation in these two layers under current treatment. Leaf photosynthesis and growth decreased as it took up more soil water from the 20−40 cm layer, whereas increasing water uptake from the 80−140 cm layer was helpful for maize leaf development under eCO_2 treatment. Promotion of water contribution from the deep soil layer increased maize photosynthesis and growth under eCO_2, which was consistent with the positive effects under the current treatment (Table 2). It was evident that the soil moisture in the deep layer could stimulate leaf growth and photosynthesis regardless of whether it suffered from current or elevated CO_2 concentrations. However, no correlation was found between WUE_L and soil water contributions in each layer under eCO_2 treatment.

4. Discussion

4.1. Impacts of eCO_2 on Crop Water Uptake

Our results demonstrated that increased CO_2 concentration to 700 ppm shifted the water uptake depth of maize (Figure 6). Water uptake patterns were closely linked to *LAI*, transpiration, photosynthesis, and water use efficiency at the leaf scale. However, these correlations exhibited significant differences in each soil layer among the eCO_2 and current treatments (Table 2). Seasonal variation in root water uptake is mainly determined by the distribution of water availability in the soil profile and crop characteristics [4,24,25,37]. Root biomass in the upper 80 cm depths was reported to account for approximately 90% of the total root biomass for maize crop [24]. It could be found that soil water in the 0−80 cm layer contributed a mean of 64.7% to maize under the current treatment over the growth period in this study. However, maize sourced significantly more water from the 0−80 cm layer particularly at the seedling and jointing growth stages under eCO_2 treatment, suggesting eCO_2 could stimulate higher carbon levels available for root growth as leaf photosynthesis rates reduced [38,39].

Shifts in root water uptake patterns due to eCO_2 typically depend on the growth stage [31]. For the current treatment at the seedling stage, roots mainly took up soil water from the surface layer (0−20 cm) [23,24,26]. Nevertheless, soil water in the 20−40 cm layer was primarily absorbed by maize at this stage under eCO_2. The reason might be that the soil evaporation in the surface layer was enhanced, indicated by the enriched isotopic values of surface soil water. This stronger evaporation resulted in greater soil water depletion in the 0−20 cm layer. Plants could increase their carbon allocation to roots extending to deeper wet soil in case of notable water depletion at the shallow depth [1]. Consequently, maize shifted to predominantly access water in the 20−40 cm layer at the seedling stage under eCO_2. The greater soil water contributions at the main water uptake depth at this stage coincided with the initial growth of maize either under current or eCO_2 treatment. Therefore, the soil water contributions had negative correlations with T_r, P_n and *LAI* at the seedling stage under both current and eCO_2 treatments.

Maize under eCO_2 became more reliant on soil water in the 40−80 cm layer when it developed into the jointing stage compared to the current treatment (Figure 6). This implied that eCO_2 induced greater root biomass accumulation in this layer to tap more soil water at the jointing stage. Furthermore, soil water holding capacity in the 40−80 cm layer was increased, associated with significant higher soil moisture ($p < 0.05$) at the tasseling stage under eCO_2 than that under the current treatment. It consequently raised the soil water contribution in the 40−80 cm layer at the tasseling stage. However, excessive water depletion in this layer reduced the T_r, P_n and *LAI* due to eCO_2.

At the latter growth stages, crops growing in an environment with high CO_2 concentration had more photosynthates allocated to the fine root growth [39–41]. The fine root biomass in the 80−140 cm layer was likely to be promoted by eCO_2 and became more efficient in absorbing soil water for increasing leaf photosynthesis and *LAI* at the filling stage (Figure 6 and Table 2). Since root water uptake is mostly driven by crop transpiration, it seemed that the consecutive reduction in T_r throughout the seedling to filling growth period restricted the root foraging capacity for accessing water from the deep soil layer of 140–250 cm (Figure 6). However, this deep-water source played an important role in increasing the T_r, P_n and *LAI* of maize both under current and eCO_2 treatments (Table 2). Plants prefer deep water sources that are hydraulically more difficult but stable to access—especially under drought conditions [9,10].

4.2. Implications of this Study

The eCO_2 decreased T_r by 17.0% but increased evapotranspiration by 2.7% at the seedling growth stage in this study. It suggested that most of the potential water savings under eCO_2 could be lost by an increase in evaporation [33,42]. Improvement of water management at this vegetative stage is critical for crop development under eCO_2 [33,43]. Straw mulching traditionally used to reduce the evaporative water loss is recommended for improving maize growth under eCO_2. Many experiments showed that eCO_2 improved C4 plant–water relations and thereby indirectly enhanced photosynthesis, growth, and yield under drought [8,11,18]. Dry conditions could induce greater root growth accessing soil water stored in deep layers to improve grain filling of maize [44].

Our experiments suggested that deficit irrigation is better applied at the jointing stage to reduce root biomass accumulation in the middle soil layer (40−80 cm). Nevertheless, greater water supply is required at the grain filling stage under eCO_2, leading to greater root growth and water uptake ability from deeper layers (80−250 cm). Crop yield responds strongly to even small amounts of additional soil water in the deep layer because it coincides with grain development [2,45]. Moreover, physiological processes such as photosynthesis may be prolonged and thus lead to greater crop growth. These implications provide important insights into agricultural water management under climate change and water scarcity environments.

The development of rooting depth was not observed during the experiments, which requires further investigation by microlysimeters in future studies. The inter-relationships between root water uptake and crop growth under current and eCO_2 treatments also need further analysis by a quantitative biophysical model. It should be noted that some environmental conditions (i.e., light intensity and wind speed) of the controlled indoor experiments in this study could not be the same as the surroundings of the natural environment, because of the technical difficulties and expensive costs. The indoor experiments are advantageous in comparing the responses of the water cycle and crop growth to different climate conditions such as increasing CO_2 and air temperature. More controlled experiments with different CO_2 concentrations are needed in future studies. The parameters and mechanisms obtained in the experiments can be incorporated into the models to predict the future grain yield under changing environments.

5. Conclusions

In this study, seasonal responses of root water uptake patterns to eCO_2 (700 ppm) and their correlations with growth of maize were determined using a device combined with a climate chamber and weighing lysimeters. The decreases in leaf transpiration and photosynthesis due to eCO_2, stimulated roots to absorb significantly more soil water from the upper 80 cm layer in the entire growing season, even from the 80−140 cm layer at the filling growth stage. However, the root water forging capability from the deep layer of 140−250 cm was reduced under eCO_2. The shifts of root water uptake sources due to eCO_2 depended on the growth stage. The main water uptake depth at the seedling stage shifted from 0−20 cm to 20−40 cm by eCO_2, when the soil water contributions were negatively correlated with LAI, T_r and P_n either under current or eCO_2 treatment. Although the soil water contribution in the 40−80 cm layer was higher at the jointing (22.7%) and tasseling (15.4%) stages under eCO_2, no correlation was found between it and leaf growth compared to the positive relations under the current treatment. Soil water extraction from the 80−140 cm layer was beneficial for the growth of LAI, T_r and P_n under eCO_2. Overall, the WUE_L increased significantly but it was not ascribed to the water contribution in an individual soil layer under eCO_2. These shifts of crop water uptake patterns by eCO_2 suggested that agricultural management practices should be applied for conserving soil water at the initial growth stage of maize under eCO_2. Increasing the water uptake capability of roots in the deep layer will provide benefits by improving the grain yield of maize.

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