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Does the Short Term Fluctuation of Mineral Element Concentrations in the Closed Hydroponic Experimental Facilities Affect the Mineral Concentrations in Cucumber Plants Exposed to Elevated CO₂?

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Does the short-term fluctuation of mineral element concentrations in the closed hydroponic experimental facilities affect the mineral concentrations in cucumber plants exposed to elevated CO₂?

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Abstract

Aims Studies dealing with plants' mineral nutrient status under elevated atmospheric CO₂ concentration (eCO₂) are usually conducted in closed hydroponic systems, in which nutrient solutions are entirely renewed every several days. Here, we investigated the contribution of the fluctuation of concentrations of N ([N]), P ([P]), and K ([K]) in nutrient solutions in this short period on their concentrations in cucumber plants exposed to different [CO₂] and N levels.

Methods Cucumber (*Cucumis sativus* L.) plants were hydroponically grown under two [CO₂] and three N levels. [N], [P], and [K] in nutrient solutions and cucumber plants were analyzed.

Results The transpiration rate (Tr) was significantly inhibited by eCO₂, whereas Tr per plant was increased due to the larger leaf area. Elevated [CO₂] significantly decreased [N] in low N nutrient solutions, which imposed an additional decrease in [N] in plants. [P] in nutrient solutions fluctuated slightly, so the change of [P] in plants might be attributed to the dilution effect and the demand change under eCO₂. [K] in moderate and

high N nutrient solutions were significantly decreased, which exacerbated the [K] decrease in plants under eCO₂.

Conclusions The short-term fluctuation of [N] and [K] in nutrient solutions is caused by the asynchronous uptakes of N, K, and water under eCO₂, which has an appreciable influence on [N] and [K] in plants besides the dilution effect. This defect of the closed hydroponic system may let us exaggerate the negative impact of eCO₂ itself on [N] and [K] in plants.

Keywords Nitrogen concentration · Phosphorous concentration · Photosynthesis · Potassium concentration · Transpiration · Water consumption

Abbreviations

ANOVA	Analysis of variance
aCO ₂	Ambient atmospheric CO ₂ concentration
DAT	Days after transplanting
DW	Dry weight
eCO ₂	Elevated atmospheric CO ₂ concentration
LED	Light-emitting diode

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LSD	Least-significant difference
OTC	Open-top chamber
Pn	Net photosynthetic rate
PVC	Polyvinyl chloride
R/S	Root to shoot dry weight ratio
SLW	Specific leaf weight
Tr	Transpiration rate

Introduction

Due to the anthropogenic activities since the preindustrial era, atmospheric CO₂ concentration ([CO₂]) has increased rapidly from 280 μmol·mol⁻¹ in 1750 to more than 400 μmol·mol⁻¹ recently (National Oceanic and Atmospheric Administration 2020). It will continue to rise and reach 800 μmol·mol⁻¹ by the end of this century if the emission of CO₂ is not well controlled (Meinshausen et al. 2011). CO₂ is a crucial component of plant photosynthesis, so elevated [CO₂] (eCO₂) can not only promote the net photosynthesis rate but also depress the transpiration rate (Lawlor and Mitchell 1991; Rogers and Dahlman 1993). Consequently, the uptake, utilization, and allocation of the mineral elements by plants are dramatically influenced by eCO₂, resulting in a significant change in mineral element concentrations in plants (Dong et al. 2018; Jin et al. 2019; Taub and Wang 2008).

Meta-analysis studies have reviewed that the concentrations of three major nutrient elements in plants, i.e., nitrogen (N), phosphorus (P), and potassium (K), were all decreased by 7~15%, 1~7.5%, and about 10%, respectively (Cotrufo et al. 1998; Deng et al. 2015; Dong et al. 2018; Loladze 2002; Sardans et al. 2017). Some possible mechanisms for this phenomenon have been proposed. Firstly, the dilution effect by the accumulation of non-structural carbohydrates is the most frequently documented hypothesis for the reduction of mineral concentrations under eCO₂ (Gifford et al. 2000; Taub and Wang 2008). Secondly, the stomatal conductance is significantly decreased when the plants are exposed to eCO₂, followed by the decrease in both transpiration and mass flow, so the mineral elements absorbed by roots and transported to above-ground parts are limited (McDonald et al. 2002; McGrath and Lobell 2013). Thirdly, concerning N, decreased N demands due to improved N use efficiency and inhibited photorespiration-dependent NO₃⁻ assimilation are

another two key factors affecting the N concentration ([N]) in plants under eCO₂ (Bloom et al. 2010; Gifford et al. 2000; Stitt and Krapp 1999).

For dealing with plants' mineral nutrient status under eCO₂, closed hydroponic experiments are usually conducted due to their accurate nutrient inputs and less interference from soil and microorganisms (Gruda 2019). In many studies, classical nutrient solution formulations are widely used, such as Hoagland and Arnon, Puppo and Rigaud, and Yamazaki nutrient solution. They were entirely renewed with an update period of 2 to 7 days in many experimental trials (Asensio et al. 2015; Bloom et al. 2010; Jauregui et al. 2017; Li et al. 2019; Ma et al. 2018; Niu et al. 2013; Rodriguez-Hernandez Mdel et al. 2014; Vicente et al. 2017; Yilmaz et al. 2016). The concentrations and proportions of mineral elements in the classical nutrient solution formulations are usually optimized based on the water and mineral demands of plants grown in ambient [CO₂] (aCO₂) condition, so that they could maintain nearly invariant levels for several days (Hoagland and Arnon 1938; Yamazaki 1982). However, when plants are exposed to eCO₂, their demands for water and each mineral nutrient are quite different from those under aCO₂ due to the depressed transpiration (Houshmandfar et al. 2018; McGrath and Lobell 2013) and the change of metabolic process (Gifford et al. 2000; Rogers et al. 1993; Taub and Wang 2008; Yi et al. 2020). Conceivably, the concentration of each mineral element in the nutrient solution will not stay at the initial value until complete replacement, which may, in turn, have a significant impact on its concentration in plants under eCO₂.

For example, it has been frequently reported that both the N demands and transpiration were decreased under eCO₂ (Cotrufo et al. 1998; Deng et al. 2015; McGrath and Lobell 2013; Taub and Wang 2008). If water consumption is decreased more than N consumption, [N] in the nutrient solution will decline gradually. The longer the solution replacement interval lasts the lower the N remains, which could directly aggravate the N deficiency in plants under eCO₂. On the contrary, the decline in plant [N] may be alleviated due to the accumulation of N in the nutrient solution as the experiment progressed. Moreover, in these studies mentioned above, plants were all grown in small containers with limited nutrient solutions (0.25 to 2.7 L plant⁻¹), which may cause a considerable fluctuation of nutrient concentrations. It is in urgent need to check whether the mineral element

concentrations will fluctuate under $e\text{CO}_2$ due to the defect of the closed hydroponic experimental facilities. Moreover, it is interesting to know whether the short-term fluctuation within the uptake duration of nutrient solutions could regulate the mineral concentrations in plants under $e\text{CO}_2$. Both have received little attention in previous studies.

N is an essential nutrient that is often limiting plant growth in natural environments (Lambers et al. 2008). N deficiency could accelerate the photosynthetic acclimation, premature senescence, and quality deterioration in plants grown under $e\text{CO}_2$ (Agüera and De la Haba 2018; Stitt and Krapp 1999). Therefore, increasing the N supply to keep step with carbon (C) inputs and coordinating the C to N ratio are recommended to alleviate or counteract the negative impact of $e\text{CO}_2$ on plants (Halpern et al. 2019; Pettersson and McDonald 1994; Sanz-Saez et al. 2010). Thus, we hypothesized that N, P, K, and water consumptions would be dramatically changed in plants grown under $e\text{CO}_2$ conditions compared with that under $a\text{CO}_2$ conditions in different N supplies. Therefore, the consumption change would result in a significant fluctuation of mineral concentrations in the nutrient solution followed by the considerable impact on their concentrations in plants. In the present study, a hydroponic experiment on cucumber plants was conducted with two $[\text{CO}_2]$ and three N levels. Four liters of the nutrient solution for one plant was entirely renewed every 4 to 7 days. The concentrations of N, P ([P]), and K ([K]) in the residual nutrient solution and cucumber plants, net photosynthesis rate, and transpiration rate were measured. The objectives of this study were (1) to characterize the consumptions of N, P, K, and water by cucumber plants under different $[\text{CO}_2]$ and N supply conditions, and (2) to assess the contribution of the short-term fluctuation of [N], [P], and [K] in the nutrient solution on their concentrations in cucumber plants.

Materials and methods

Plant culture

Pot experiments were conducted in the glasshouse at Institute of Soil Science, Chinese Academy of Sciences, Nanjing, P.R. China (32.0596° N, 118.8050° E). Cucumber (*Cucumis sativus* L.) seeds of cv. 'Jinyou 38' (Tianjin Lv Feng Co., Ltd., Tianjin, China) were surface sterilized with 10% sodium hypochlorite for 15 min,

washed with ultra-pure water thoroughly, and then germinated on moist filter paper in a growth chamber at 28 °C and relative humidity of 70%. Three days later, the seeds with radicles were sown into trays containing peat-vermiculite mixture (2:1, v/v) substrate. After 2 weeks, seedlings with three fully expanded true leaves were transplanted to polyvinyl chloride polymer (PVC) pots with 4 L of nutrient solution and two plants per pot, under $a\text{CO}_2$ conditions. Eleven days after transplanting (DAT), seedlings with five fully expanded true leaves were transplanted to PVC pots containing 4 L nutrient solution with one plant per pot and transferred into two open-top chambers (OTCs) (2.3 m length \times 0.8 m width \times 1.4 m height) with natural solar radiation. Individual cucumber plants were inserted into the hole on the cover of PVC pots, held with black foam sleeves, sealed with aluminum foil tape to minimize the solvent evaporation, and hung with wires to keep vertical growing.

The experiment was carried out as a split-plot design with $[\text{CO}_2]$ as the primary treatment and N supply as the sub-plot treatment. Three N treatments were set at 7 (low N, N1), 14 (moderate N, N2), and 21 (high N, N3) mM, which was repeated in four pots in each chamber. Twelve pots in each chamber were rotated within and among chambers every week to reduce the chamber and positional effects. The $[\text{CO}_2]$ and N supply treatments were implemented from 12 until 44 DAT. During this period, the $[\text{CO}_2]$ in OTCs was controlled at either $a\text{CO}_2$ (hereafter, also referred to as the C1 or 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ treatment), or $e\text{CO}_2$ (the C2 or 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ treatment). Carbon dioxide concentrations in the chambers were monitored every 5 min between 0900 and 1600 h each sunny and cloudy day using an infrared gas analyzer (Ultramat 6, Siemens, Munich, Germany). The accumulated CO_2 treating time was 189 h in the whole experiment, and the average $[\text{CO}_2]$ achieved were 397.1 ± 5.9 and 807.0 ± 7.8 $\mu\text{mol}\cdot\text{mol}^{-1}$ (means \pm SD) for the $a\text{CO}_2$ and $e\text{CO}_2$ treatments, respectively (Fig. S1). According to the data recorded by a L95–83 data logger (Hangzhou loggertech Co., Ltd., Hangzhou, China) every 15 min, the average temperature in OTCs was 23.7 ± 3.3 °C (C1) and 24.1 ± 3.0 °C (C2), and the average humidity was $56.3 \pm 9.7\%$ (C1) and $58.6\% \pm 10.2\%$ (C2), respectively.

Yamazaki nutrient solution for cucumber (Yamazaki 1982) was used as the moderate N treatment (N2), and was modified to create the low N (N1) and high N (N3) treatments (Table 1). To ensure the same $\text{NH}_4^+:\text{NO}_3^-$

ratios, and P, K, and Mg concentrations in all three N treatments, anions and cations were balanced with SO_4^{2-} and Ca^{2+} , respectively. Micro-nutrients were included as follows: ($\text{mg}\cdot\text{L}^{-1}$): $\text{Na}_2\text{Fe}\cdot\text{EDTA}$ (29.27), H_3BO_3 (2.86), $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$ (2.03), $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ (0.22), $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (0.08), and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (0.02). The pH of the nutrient solutions was adjusted to pH 6.5 by adding diluted NaOH. A bacteriostat (Micropur, $1.0\text{ mg}\cdot\text{L}^{-1}$; Siches Trinkwasser, Germany) was added to inhibit the activity of microorganisms (Amann and Amberger 1989). The solutions were aerated for 30 min every hour and were completely replaced on 11, 16, 22, 26, 31, 37, and 44 DAT (Fig. S1). The old solutions were retained for subsequent analysis of nutrient depletion.

Determination of leaf gas exchange parameters

The gas exchange measurements of cucumber plants were evaluated on six fully expanded leaves from individual cucumber plants from 0900 to 1500HR on 44 DAT. The net photosynthetic rate (Pn) and transpiration rate (Tr) of cucumber plants were measured by a portable photosynthesis system (Li-6400, Li-Cor Inc., Lincoln, OR, USA) with a standard leaf chamber ($2\text{ cm}\times 3\text{ cm}$) (6400-02B) and a LED light source. The $[\text{CO}_2]$ of the flow-in air was set to the same level as the relevant treatment. The photosynthetic photon flux density, temperature, relative air humidity, and the air-flow rate inside the leaf chamber were set at $1500\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $25\text{ }^\circ\text{C}$, 50%, and $500\text{ }\mu\text{mol}\cdot\text{s}^{-1}$, respectively.

The residual nutrient solution collection and analysis

The residual nutrient solution in the pots was collected before replacement on the same day when the fresh nutrient solution was added. They were suction-filtered through $0.45\text{-}\mu\text{m}$ Millipore membrane (Merck Millipore, Billerica, MA, USA) and weighed immediately after collection, and 200 mL of each filtrate was stored at $-18\text{ }^\circ\text{C}$ for further determination. The NO_3^- and NH_4^+ concentrations in the residual nutrient solutions were determined with an automatic continuous segmented flow analyzer (SAN++, Skalar Analytical B.V., Breda, The Netherlands). The PO_4^{3-} and K^+ concentrations in the residual nutrient solutions were quantified using an inductive coupled plasma optical emission spectrometry (Optima 8000, PerkinElmer, Waltham, MA, USA). [N] in the residual nutrient solution

was calculated as the sum of NO_3^- and NH_4^+ concentrations. [P] and [K] were represented by the PO_4^{3-} and K^+ concentrations, respectively.

Plant harvest and analysis

Cucumber plants were harvested on 44 DAT immediately after the gas exchange measurements. The plants were separated to root, stem, leaf, and fruit samples and washed with tap water followed by distilled water. Dry weight (DW) of each organ was obtained by drying the fresh samples at $105\text{ }^\circ\text{C}$ for 30 min and then at $75\text{ }^\circ\text{C}$ to a constant weight in an electro-thermostatic oven. All the fresh leaves on the individual plants were stacked together, and the leaf numbers were recorded. Fresh leaf discs (12-mm diameter) were cut by a hole-punch and placed into aluminum cups, then dried at $75\text{ }^\circ\text{C}$ to a constant weight. Specific leaf weight (SLW) was determined as the ratio of dry weight to the total area of leaf discs. Based on the SLW and DW of leaves, the total leaf area of individual plants was calculated. Dry samples were ground to pass through a 0.5-mm sieve, and 200 mg of each sample was soaked in 5 mL concentrated H_2SO_4 for 24 h then digested at $180\text{ }^\circ\text{C}$ for 5 h, followed by intermittent addition of 0.5 mL H_2O_2 for 2 to 3 times. The extracted solution was diluted with deionized water, and [N] was determined using a colorimetric method based on the salicylate-chlorine reaction and absorption spectrometry with sodium nitroprusside and sodium dichloroisocyanurate by an automated Discrete Chemistry Analyzer (Smartchem 200, Westco Scientific Instruments Inc., Rome, Italy) (Reardon et al. 1966). Another portion of 200-mg dry samples was digested with 5 mL $\text{HNO}_3\text{-HClO}_4$ (85:15 v/v) at $190\text{ }^\circ\text{C}$, and [P] and [K] were analyzed by an inductively coupled plasma atomic optical emission spectrometer (ICP-OES) (Optima 8000, PerkinElmer, Boston, USA) (Zhao et al. 2008).

Statistical analysis

Statistical analysis and figures were generated by OriginPro (Version 2019b; OriginLab Corp., Northampton, MA, USA). All data were shown as mean \pm standard error. The means of DW, gas exchange properties, mineral concentrations, consumptions, and accumulations with four replicates in each treatment were compared using Fisher LSD test at a significance level of $p = 0.05$ by using the application “Paired Comparison Plot” in OriginPro. The effects of N supply level and $[\text{CO}_2]$, and their interaction on DW, gas exchange

Table 1 Components of macro-nutrient elements in solutions with different N levels. -, no chemicals

N levels	Compounds (mmol L ⁻¹)						Elements (mmol L ⁻¹)							
	Ca(NO ₃) ₂ ·4H ₂ O	KNO ₃	NH ₄ NO ₃	MgSO ₄ ·7H ₂ O	K ₂ SO ₄	Ca(H ₂ PO ₄) ₂ ·H ₂ O	N			P	K	Ca	Mg	S
							Total	NH ₄ ⁺	NO ₃ ⁻					
N1	3	-	0.5	2	3	0.5	7	0.5	6.5	1	6	3.5	2	5
N2	3	6	1	2	-	0.5	14	1	13	1	6	3.5	2	2
N3	6	6	1.5	2	-	0.5	21	1.5	19.5	1	6	6.5	2	2

properties, mineral concentrations, consumptions, and accumulations were quantified using two-way analysis of variance (ANOVA) at a significance level of $p = 0.05$. Linear regression analyses and significance tests were performed using the application “Simple Fit” (a linear model function) in OriginPro. The 95% confidence interval for the regression slope was also constructed in the figures.

Results

The growth and gas exchange properties of cucumber plants

The growth parameters of cucumber plants in response to [CO₂] and N supply levels are shown in Fig. 1. Two-way ANOVA results showed a significant effect of [CO₂] and N supply levels on the DW formation of cucumber plants. The root, stem, leaf, fruit, and total DW were significantly higher under eCO₂ than those under aCO₂ condition in high N supply (N3), whereas the increase of aerial part DW in low N supply (N1) was not significant. Averagely, the increase of total DW was 39.6% (not significant = n.s.), 76.2%, and 84.3% by eCO₂ in N1, N2, and N3 supply, respectively. Meanwhile, the increase of DW in different organs by higher N supply was dramatic under eCO₂ but not noticeable under aCO₂. Compared with the low N treatment, the total DW was 71.6% and 126.6% higher in the high N treatment under aCO₂ and eCO₂, respectively. Increasing the N supply resulted in a significant decrease of root/shoot DW ratio (R/S), whereas the R/S was less affected by eCO₂ regardless of N supply. SLW was 23.9% higher under eCO₂ than that under aCO₂ only in high N supply and was 17.5% higher in high N supply than that in low N supply only under eCO₂ condition.

Like the leaf DW, a significant increase of the leaf area per plant was observed by eCO₂ in moderate (N2) and high (N3) N supply, and the increase was 117.3% and 64.4%, respectively. Moreover, the increase of the leaf area per plant by increasing N supply was significant only under eCO₂ condition and was 57.2% and 70.7% in N2 and N3 treatment compared with N1 treatment, respectively.

As shown in Fig. 2, the gas exchange rates measurements indicated that the Pn of cucumber plants grown under eCO₂ was 1.91- to 2.12-fold higher than that under aCO₂ condition. Due to the larger leaf area per plant under eCO₂ condition, the Pn per plant was much larger under eCO₂ than that under aCO₂. The Pn per plant under eCO₂ was 2.61, 4.97, and 3.25 times as much as those under aCO₂ condition in N1, N2, and N3 treatment, respectively. Under eCO₂, the Pn per plant was increased by 87.2% and 95.2% in the moderate and high N treatment compared with that in the low N treatment, respectively. The Tr of cucumber plants was significantly inhibited by eCO₂ in low and moderate N supplies but only had a declining trend in high N supply. Generally, the Tr under eCO₂ was 48.1%, 35.2%, and 29.9% (n.s.) lower than those under aCO₂ condition, in N1, N2, and N3 treatment, respectively. Concerning Tr per plant, larger leaf area per plant counteracted the decrease of Tr under eCO₂ condition. Therefore, Tr per plant under eCO₂ has little difference with or even larger than that under aCO₂ due to the larger leaf area per plant. The increase of Tr per plant by eCO₂ was 59.4% and 39.7% (n.s.) in the moderate and high N treatment, respectively.

The fluctuation of [N], [P], and [K] in nutrient solutions

Figure 3 shows the fluctuation of [N] in six rounds of nutrient solution treatments with three N supply levels,

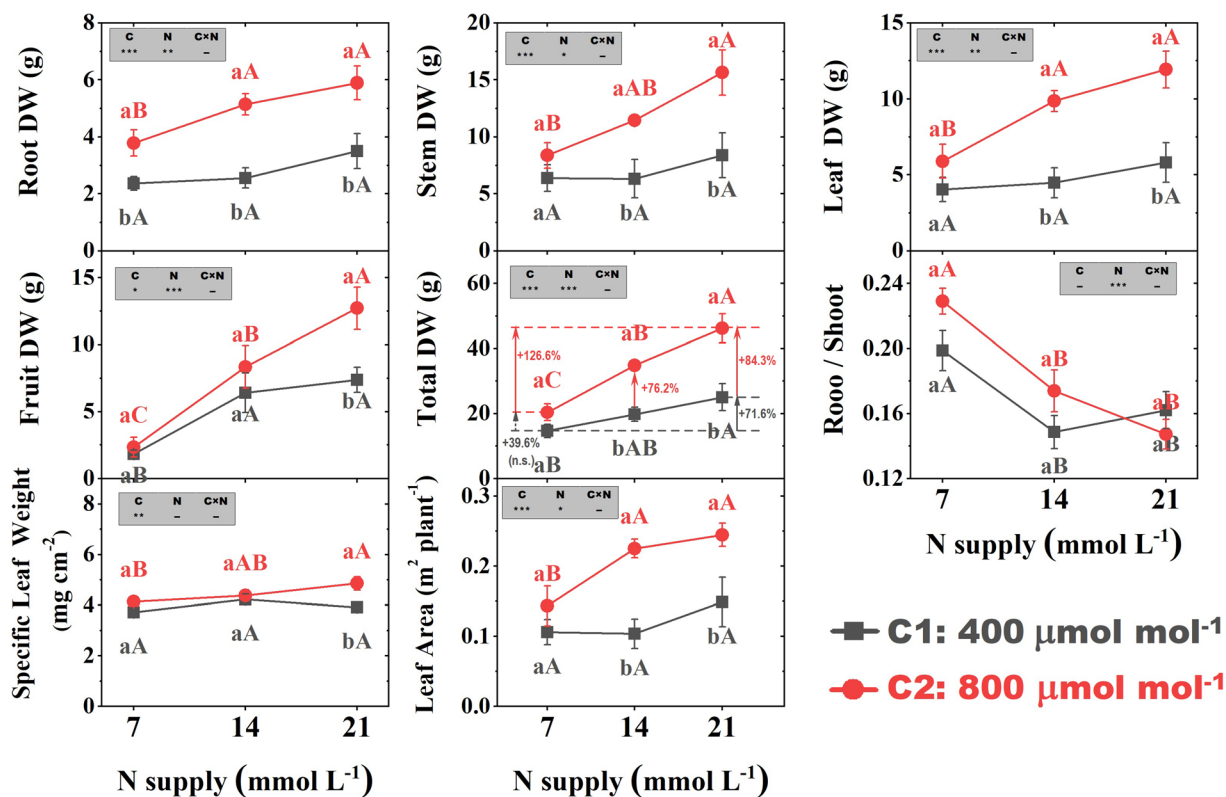


Fig. 1 Dry weights (DW) of roots, stems, leaves, fruits, whole plants, root/shoot ratios, specific leaf dry weights, and leaf areas of cucumber plants grown under different CO₂ and N levels ($n = 4$). Bars represent standard errors. Means not followed by the same lower case letters are significantly different among different CO₂ levels in the same N level, and not followed by the same upper

case letters are significantly different among different N levels in the same CO₂ level, according to Fisher LSD test at $p < 0.05$. In the internal table, C, CO₂ level; N, N level. Asterisks (*) indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); Hyphen (-) indicates non-significant differences ($p \geq 0.05$)

from which a general dilution of [N] during the plant growth was found. [N] in the residual nutrient solution under eCO₂ was lower than that under aCO₂ condition. The decrease was significant in the first (from 11 DAT to 16 DAT), the fourth (from 26 DAT to 31 DAT), and the fifth (from 31 DAT to 37 DAT) round of nutrient solution treatments in all three N levels. It is noteworthy that [N] in the residual nutrient solution of N1 treatments were less than 4 mM or even 2 mM in the first three rounds. Although the decrease by eCO₂ was significant in all the six rounds in N3 treatments, [N] in the six residual nutrient solutions were still more than 7 mM.

[P] in the residual nutrient solution was also lower than its initial concentration in the fresh nutrient solution (Fig. 4). The effects of [CO₂] on [P] in the residual nutrient solution depended on the N supply levels and growth stages. In the first four rounds of nutrient solution treatments (from 11 DAT to 31 DAT), [P] in the residual

nutrient solution was significantly lower under eCO₂ than that under aCO₂ condition in all three N supply levels. The average decrease was 19.3%, 19.3%, and 17.8% for N1, N2, and N3, respectively. In the last two rounds of nutrient solution treatments (from 31 DAT to 44 DAT), compared with that under aCO₂ condition, [P] in the residual nutrient solution under eCO₂ condition was decreased by 22.5% and 22.3% in N1 and N2 respectively, but stayed the same in N3. The N supply levels also had a significant impact on [P] in the residual nutrient solution. [P] in the residual nutrient solution showed a steep decrease with the N supply increasing in the whole experimental period under both [CO₂] conditions. Compared with that in N1, the average decrease in N3 was 21.2% and 16.6% under aCO₂ and eCO₂ condition, respectively. Although it was always the lowest in N3 under eCO₂ condition, [P] in the residual nutrient solution ranged from 0.45 to 0.64 mM, about half of the initial value.

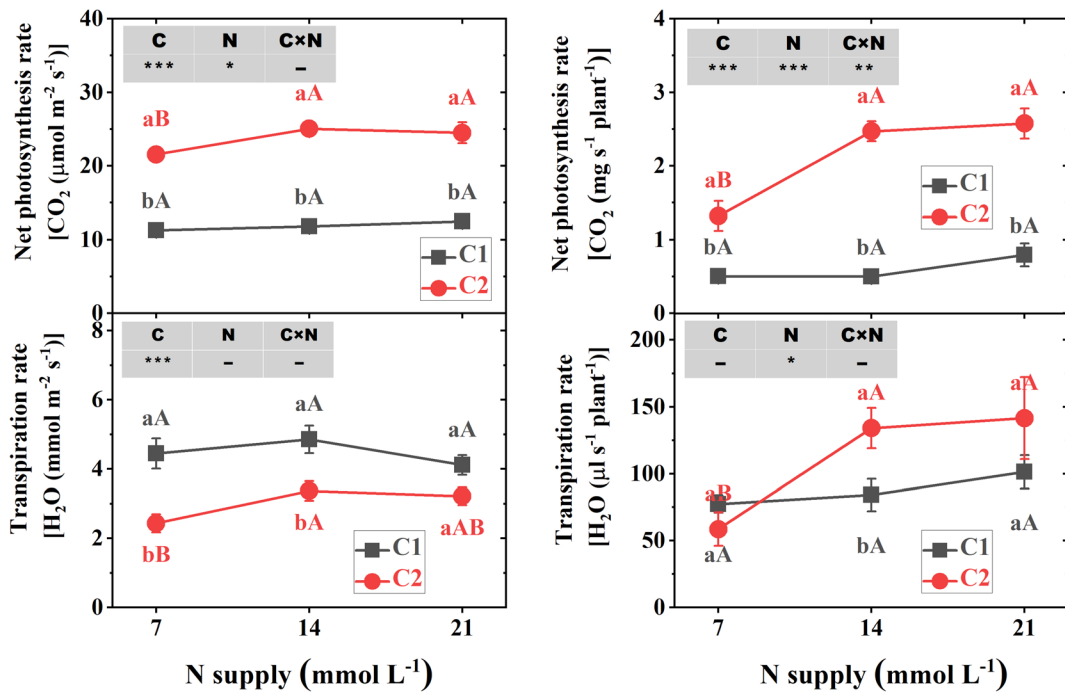


Fig. 2 Net photosynthesis rate, photosynthesis rate per plant, transpiration rate, and transpiration rate per plant of cucumber plants grown under different CO₂ and N levels (*n* = 4). Bars represent standard errors. CO₂ levels: C1, 400 μmol·mol⁻¹; C2, 800 μmol·mol⁻¹. Means not followed by the same lower case letters are significantly different among different CO₂ levels in

the same N level, and not followed by the same upper case letters are significantly different among different N levels in the same CO₂ level, according to Fisher LSD test at *p* < 0.05. In the internal table, C, CO₂ level; N, N level. Asterisks (*) indicate significant differences (**p* < 0.05; ***p* < 0.01; ****p* < 0.001); Hyphen (-) indicates non-significant differences (*p* ≥ 0.05)

[K] in the residual nutrient solution after six rounds of nutrient solution treatments is shown in Fig. 4. The final [K] was similar to its initial concentration in low N supply under aCO₂ condition but was observably lower in high N supply under eCO₂ condition. In N1 supply, no significant reduction in [K] in the residual nutrient solution was found under eCO₂ compared with that under aCO₂ condition, except for the fourth and fifth rounds (from 26 DAT to 37 DAT). In N2 and N3 supplies, [K] in the residual nutrient solution was significantly lower under eCO₂ than that under aCO₂ condition, except for the second round in N2 supply. The average decrease under eCO₂ was 21.8% and 23.6% in N2 and N3, respectively. [K] in the residual nutrient solution also exhibited a general decline with the N supply increasing regardless of [CO₂] and growth stage. The decreases from N1 to N3 were 13.7% and 27.5% under aCO₂ and eCO₂ condition, respectively. The lowest value of [K] in the residual nutrient solution in the last four rounds ranged from 3.1 to 3.8 mM.

According to the water volume, [N], [P], and [K] in the fresh and residual nutrient solution, the water, N, P,

and K consumptions in each round and their total consumptions by individual cucumber plants from the nutrient solution were calculated (Fig. 5). When the plants received moderate (N2) and high (N3) N supply, eCO₂ significantly increased the water consumption per plant by 30.1% and 27.6% compared with those under aCO₂ condition. The water consumption was dramatically increased by 75.9% with the N supply increasing from N1 to N3 under eCO₂ condition. Elevated N supply significantly increased the N consumption per plant, and the average increases were 106.9% and 188.1% in N3 compared with that in N1 under aCO₂ and eCO₂ condition, respectively. In N2 and N3 treatments, eCO₂ increased the N consumption per plant by 60.9% and 72.9%, respectively. The P consumption per plant was steadily increased with either N supply or [CO₂] increasing. The increase of P consumption per plant by eCO₂ was 60.4%, 50.2%, and 27.2% for N1, N2, and N3, respectively. Compared with that in N1, the P consumption per plant was 73.7% and 37.8% higher in N3 under aCO₂ and eCO₂ condition, respectively. The K consumption per plant was significantly increased by

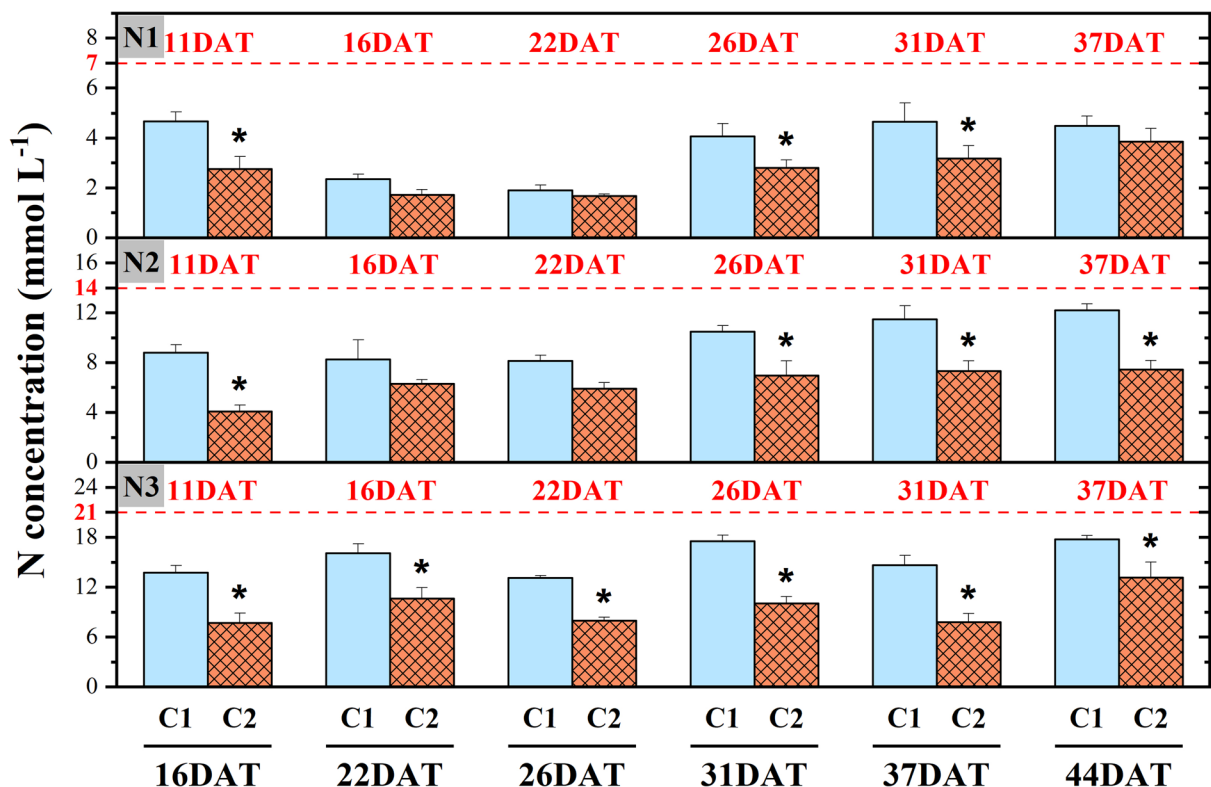


Fig. 3 The final nitrogen concentration in the residual nutrient solution on the day when the nutrient solution was renewed under different CO₂ and N levels ($n = 4$). Bars represent standard errors. CO₂ levels: C1, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$; C2, 800 $\mu\text{mol}\cdot\text{mol}^{-1}$. N levels: N1, 7 $\text{mmol}\cdot\text{L}^{-1}$; N2, 14 $\text{mmol}\cdot\text{L}^{-1}$; N3, 21 $\text{mmol}\cdot\text{L}^{-1}$. Asterisk

(* $p < 0.05$) indicates significant differences between two different CO₂ levels in the same N level on the same day. The red dotted line and date indicate the initial nitrogen concentration in the fresh nutrient solution and the date on which the nutrient solution was renewed

97.0% and 65.1% in N2 and N3 by eCO₂, respectively. The increases of the K consumption per plant from N1 to N3 were 99.1% and 122.7% under aCO₂ and eCO₂ condition, respectively.

[N], [P], and [K] and their accumulations in cucumber plants

[N], [P], and [K] in different organs of cucumber plants gave us information about mineral nutrients status in different N supplies and [CO₂] treatments (Fig. 6). There was no significant decline of [N] in all organs of cucumber associated with eCO₂ when plants received N2 or N3 supply, and the decrease was significant only in leaves (-15.2%) and fruits (-12.0%) when plants were grown in N1 supply. [N] in all cucumber plants' organs were increased from N1 to N3 in both [CO₂] treatments. There was no significant decrease in [P] in all organs of cucumber plants by eCO₂ except for the roots received N2 or N3 supply. Elevated CO₂ increased

[P] by 32.4% in stems and 29.0% in fruits received low N supply. Increasing the N supply from N1 to N3 has little effects on [P] in cucumber plants under aCO₂ but decreased [P] by 32.5%, 28.6%, and 27.7% in the roots, stems, and fruits under eCO₂ condition, respectively. There were no significant differences in [K] in all organs of cucumber plants received low N supply between two [CO₂] treatments, whereas eCO₂ dramatically reduced [K] by 20.2% in roots, 14.8% in stems, 24.3% in leaves, and 17.2% in fruits in cucumber plants received N3 supply. N supply also substantially impacted [K] in cucumber plants depending on the [CO₂] conditions. Under aCO₂ condition, [K] was decreased by 24.6% in roots and increased by 85.5% in leaves when the N supply was increased from N1 to N3. However, under eCO₂ condition, the decrease of [K] from N1 to N3 was 36.0% in roots and 19.0% in fruits.

Based on the DW and nutrient concentrations, the N, P, and K accumulations in different organs and the whole plant of cucumber grown under different [CO₂]

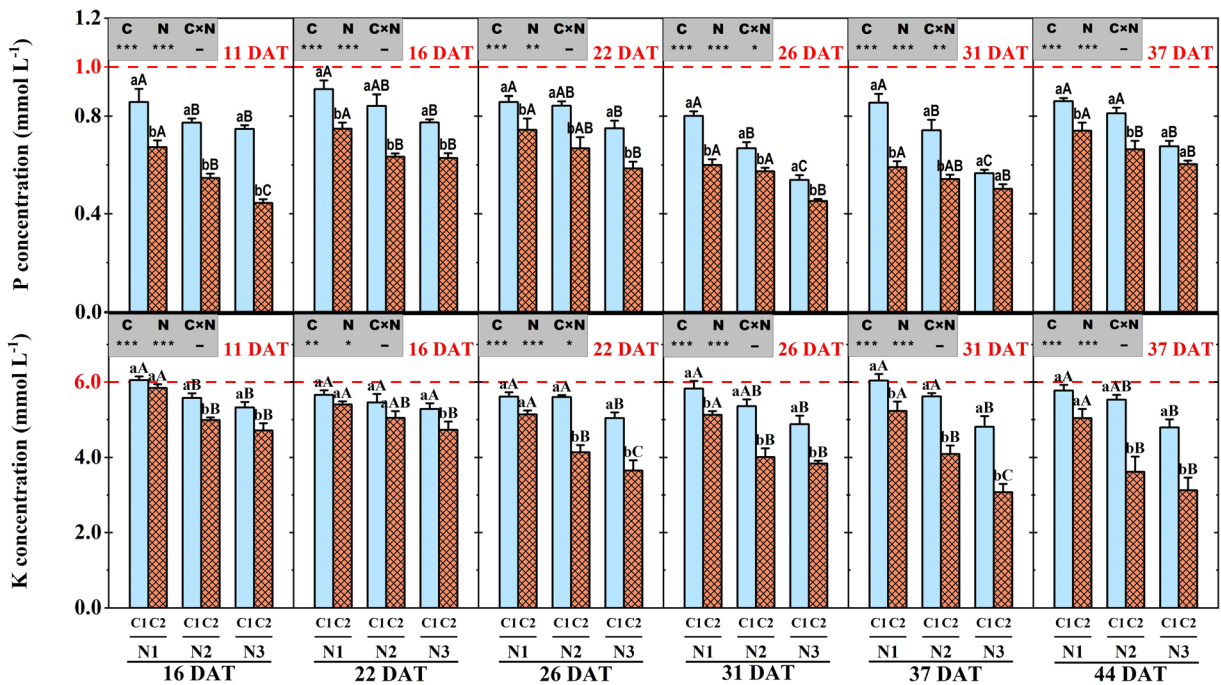


Fig. 4 The final phosphorus and potassium concentrations in the residual nutrient solution on the day when the nutrient solution was renewed under different CO₂ and N levels (*n* = 4). Bars represent standard errors. CO₂ levels: C1, 400 μmol·mol⁻¹; C2, 800 μmol·mol⁻¹. N levels: N1, 7 mmol L⁻¹; N2, 14 mmol L⁻¹; N3, 21 mmol L⁻¹. Means not followed by the same lower case letters are significantly different among different CO₂ levels in the same N level, and not followed by the same upper case letters are

significantly different among different N levels in the same CO₂ level, according to Fisher LSD test at *p* < 0.05. In the internal table, C, CO₂ level; N, N level. Asterisks (*) indicate significant differences (**p* < 0.05; ***p* < 0.01; ****p* < 0.001); Hyphen (-) indicates non-significant differences (*p* ≥ 0.05). The red dotted line and date indicate the initial phosphorus and potassium concentrations in the fresh nutrient solution and the date on which the nutrient solution was renewed

and N levels were obtained (Fig. S2). Only P accumulation was increased by eCO₂ in low N treatment, whereas the increases by eCO₂ in high N treatment were 79.8%, 78.7%, and 74.1% for N, P, and K accumulations, respectively. When the N supply was increased from N1 to N3, the N, P, and K accumulations were increased by 100.0%, 60.8%, and 64.1% under aCO₂ condition, and 192.2%, 72.2%, and 115.7% under eCO₂ condition, respectively. Significant positive correlations between mineral element accumulations per plant and the corresponding consumption from the nutrient solution in the whole experimental period were also observed, with the R² of 0.919, 0.867, and 0.937 for N, P, and K element respectively (Fig. S3).

The correlations between biomass accumulation, gas exchange properties, and water and mineral element consumptions

To further evaluate the relationship between the biomass accumulation, water consumption, and gas exchange

properties in different [CO₂] and N supply treatments, Pearson correlation coefficient was analyzed (Fig. 7). In the whole experimental period, there was a significant positive correlation between the total DW accumulation and the net photosynthesis rate per plant with the R² of 0.700. The water consumption was also positively correlated to the transpiration rate per plant (R² = 0.815). The correlations between N, P, and K consumptions and the water consumption per plant in cucumber plants were also generated by using the four replications data in six rounds of nutrient solution treatments (Fig. 7). All the N, P, and K consumptions from the nutrient solution were significantly positively correlated to the corresponding water consumption with the R² of 0.194, 0.484, and 0.452 for N, P, and K element respectively (Fig. 7). When cucumber plants absorbed a certain mass (or volume) of water, the simultaneous absorption of N was significantly increased by eCO₂ and higher N supply. The P consumption per unit water consumption was greater under eCO₂ than that under aCO₂ condition in low N and moderate N supply but not in high N supply.

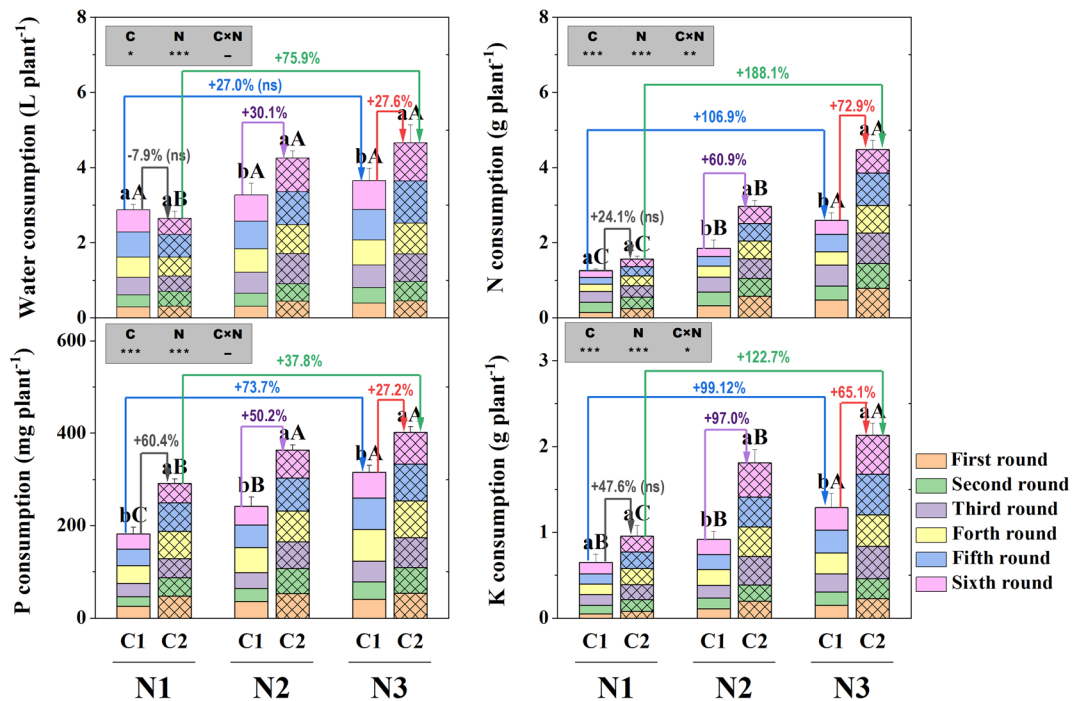


Fig. 5 The water, nitrogen, phosphorus, and potassium consumptions per plant in each nutrient solution round and the whole experimental period under different CO₂ and N levels ($n = 4$). Bars represent standard errors. CO₂ levels: C1, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$; C2, 800 $\mu\text{mol}\cdot\text{mol}^{-1}$. N levels: N1, 7 $\text{mmol}\cdot\text{L}^{-1}$; N2, 14 $\text{mmol}\cdot\text{L}^{-1}$; N3, 21 $\text{mmol}\cdot\text{L}^{-1}$. Means not followed by the same lower case letters are significantly different among different CO₂ levels in the same

N level, and not followed by the same upper case letters are significantly different among different N levels in the same CO₂ level, according to Fisher LSD test at $p < 0.05$. In the internal table, C, CO₂ level; N, N level. Asterisks (*) indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); Hyphen (-) indicates non-significant differences ($p \geq 0.05$)

Similarly, the K consumption per unit water consumption was more massive under eCO₂ than that under aCO₂ condition in N1 supply and increased with N supply increasing under aCO₂ condition. The N, P, and K consumptions from the nutrient solution also had a strong positive correlation with the Tr per plant, and the R² was highest for N (0.741) and lowest for P (0.549) (Fig. S4).

Discussion

Plant growth and gas exchange properties

The increases of the organs and total DW of cucumber plants in high N supply and eCO₂ treatments in this work are consistent with previous reports (Dong et al. 2017; Kläring et al. 2007; Li et al. 2018; Li et al. 2020; Peet 1986). Our results demonstrated that the biomass accumulation was positively correlated to the net photosynthesis rate per plant (Fig. 7), which indicated that

eCO₂ promoted the fixation of CO₂ and thus biomass accumulation under moderate or high N supply (Reich et al. 2006; van der Kooi et al. 2016). It has been reported that eCO₂ usually increases R/S in nutrient-limiting condition because the increased biomass by eCO₂ will be preferentially allocated to roots to exploit and acquire more nutrients (Rogers et al. 1994; Stulen and Denhartog 1993). In the present study, there was only an increasing trend in R/S by eCO₂ in N1 and N2 treatments (Fig. 1), which could be explained as the N1 treatment (7 mM) was not a severe N deficiency condition for cucumber growth (Dong et al. 2017). In addition, as an indicator of leaf thickness and toughness, we found that the SLW showed a 23.9% increase in N3 treatment by eCO₂ and a 17.5% increase under eCO₂ condition by raising the N supply from 7 to 21 mM. These results were related to the thickening of the leaf cell walls and the enhanced allocation of structural material per unit area of leaves under higher N and eCO₂ condition (Conroy et al. 1992; Pritchard et al. 1999).

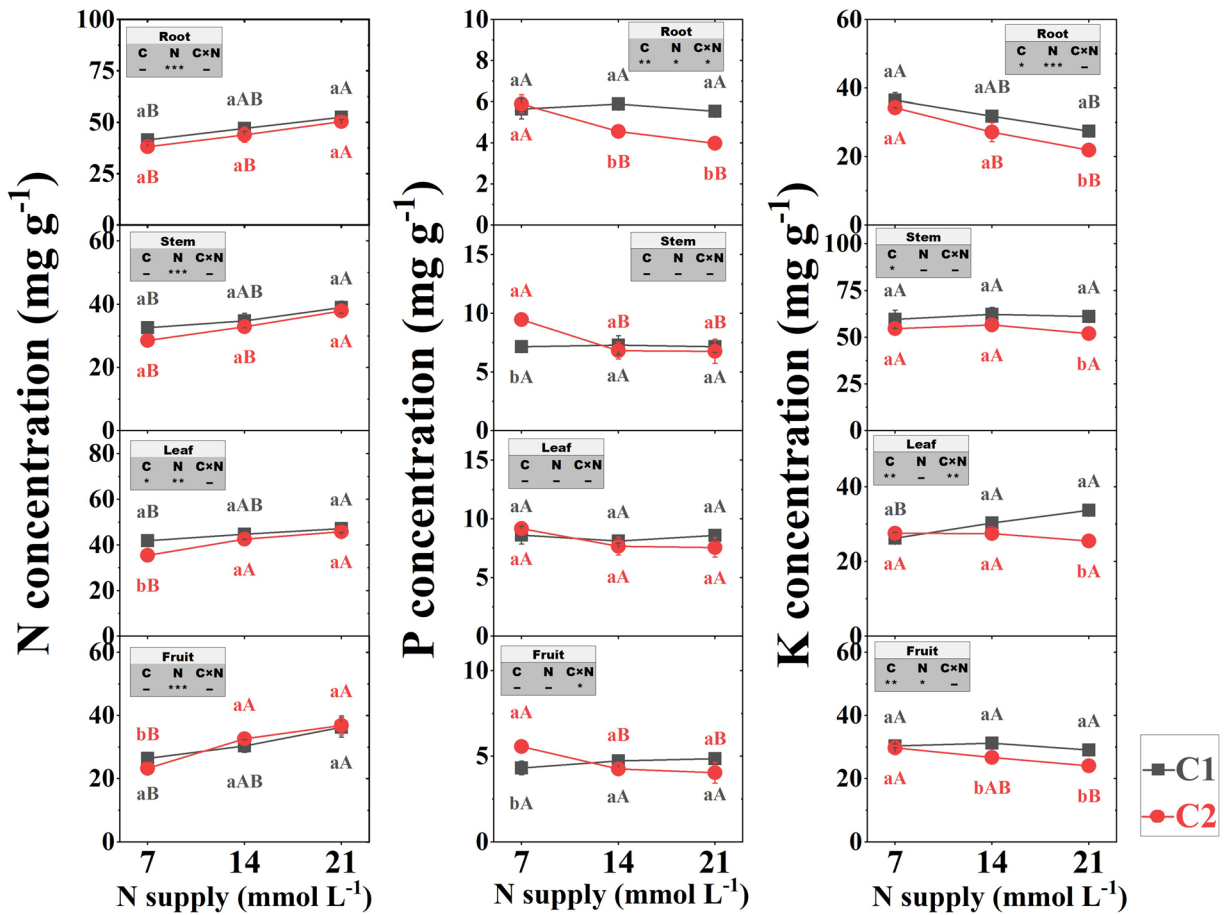


Fig. 6 The nitrogen, phosphorus, and potassium concentrations in roots, stems, leaves, and fruits of cucumber plants grown under different CO₂ and N levels (*n* = 4). Bars represent standard errors. CO₂ levels: C1, 400 μmol·mol⁻¹; C2, 800 μmol·mol⁻¹. Means not followed by the same lower case letters are significantly different among different CO₂ levels in the same N level, and not followed

by the same upper case letters are significantly different among different N levels in the same CO₂ level, according to Fisher LSD test at *p* < 0.05. In the internal table, C, CO₂ level; N, N level. Asterisks (*) indicate significant differences (**p* < 0.05; ***p* < 0.01; ****p* < 0.001); Hyphen (-) indicates non-significant differences (*p* ≥ 0.05)

It is worth noting that the Tr per plant was larger under eCO₂ than that under aCO₂ in N2 and N3 supply treatments, and it increased with increasing N supply under eCO₂, although the Tr was significantly inhibited by eCO₂ (Fig. 2). Therefore, the higher Tr per plant under eCO₂ in moderate and high N levels was likely the result of increased leaf area per plant, which ultimately offsets the lower Tr under eCO₂ (Wei et al. 2018; Yi et al. 2020). Moreover, the lower Tr per plant in N1 compared with that in N2 and N3 treatments would be ascribed to the smaller leaf area of the cucumber plants in low N conditions (Wei et al. 2018). The highly positive correlation between the water consumption and Tr per plant in the whole experimental period (Fig. 7) further indicated that Tr per plant was a good indicator for water usage status at the plant level.

As shown in Fig. S3, the accumulations of N, P, and K in plants were all smaller than their consumption from the nutrient solution. The main reason was that the amounts of N, P, and K in the leaf litter (i.e., leaves that had withered) were not included in the accumulation calculation. Besides, some N may be lost through denitrification from the nutrient solution or lost as NH₃ from stomata in leaves. So, we could find a more considerable loss in N than that in P or K.

Although the loss of N, P, and K existed, the spots presenting the observed values under different eCO₂ and N levels were all near the fit line, which means the loss was almost the same among different treatments. Moreover, the highly positive correlation (*R*² = 0.867–0.937) between mineral element accumulations per plant and the corresponding consumption from the

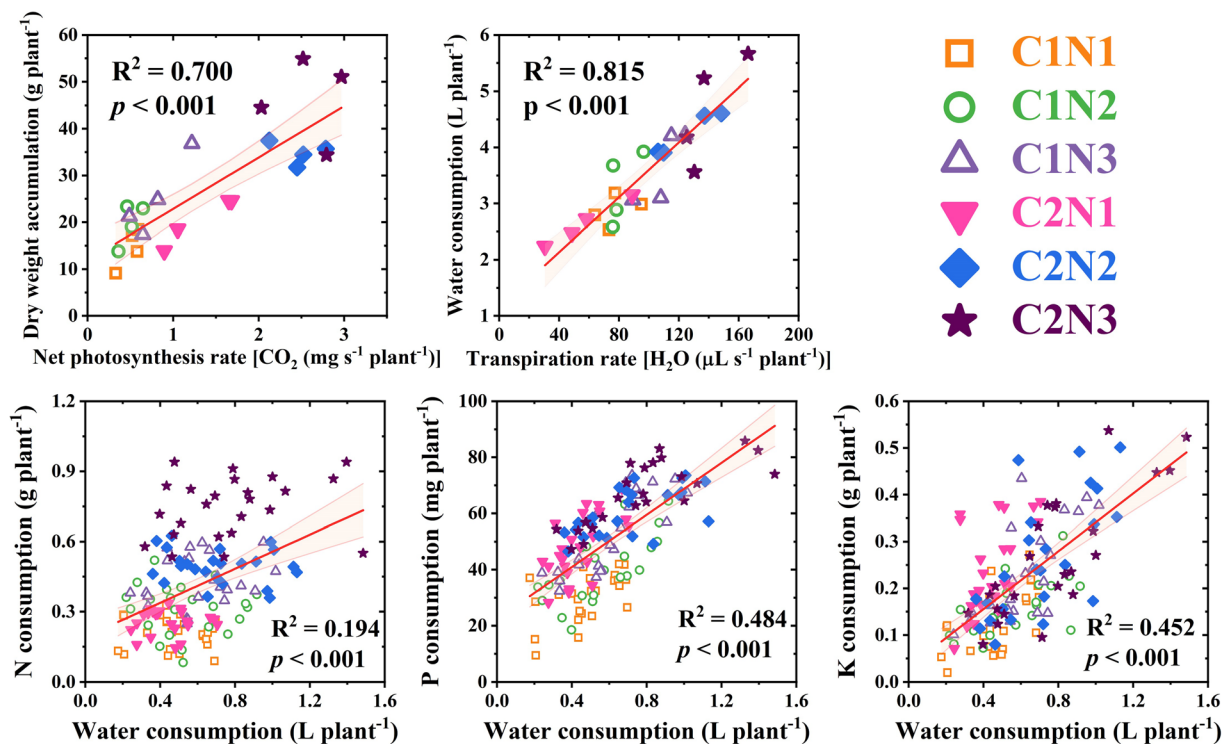


Fig. 7 The relationship between the biomass accumulation and photosynthesis rate per plant; the water consumption and transpiration rate per plant in the whole experimental period; the nitrogen, phosphorus, and potassium consumptions per plant from the nutrient solution, and the corresponding water consumption in each

nutrient solution round, respectively. CO₂ levels: C1, 400 μmol·mol⁻¹; C2, 800 μmol·mol⁻¹. N levels: N1, 7 mmol L⁻¹; N2, 14 mmol L⁻¹; N3, 21 mmol L⁻¹. Shaded area around the curves represents 95% confidence intervals

nutrient solution further indicated that the nutrient consumption was a good indicator for the nutrient accumulation at the plant level.

Nitrogen concentrations in plants

A decrease in [N] in plants under eCO₂ condition is frequently reported, and the decrease also strongly depends on the N supply levels (Gifford et al. 2000; Pettersson and McDonald 1994). Usually, the decrease in [N] by eCO₂ is more dramatic in N-deficient plants yet is mostly alleviated in N-replete plants (Rogers et al. 1994; Stitt and Krapp 1999). Moreover, it has been reviewed that the average [N] decrease in leaves (16%) is larger than those in stems (9%) and roots (9%) (Cotrufo et al. 1998; Sardans et al. 2012). In the present study, eCO₂ significantly decreased [N] in leaves and fruits in plants that received low N supply (Fig. 6), which coincided with the findings mentioned above. To interpret the reduction of [N] in plants by eCO₂, the dilution effect due to the accumulation of non-

structural carbohydrates and the reduced mass flow and root-to-shoot translocation due to decreased transpiration are two well-documented mechanisms (Gifford et al. 2000; McDonald et al. 2002; McGrath and Lobell 2013). In this study, eCO₂ increased the total DW by 39.6% (n.s.), 76.2%, and 84.3% (Fig. 1) and increased the N accumulation by 23.1% (n.s.), 78.0%, and 79.8% (Fig. S2) in N1, N2, and N3 supply, respectively. Therefore, the decrease of [N] in plants with low N supply by eCO₂ could be interpreted by the dilution effect. Although eCO₂ significantly inhibited the Tr in N1 and N2 treatments, only [N] in plants in N1 was significantly decreased. It indicated that decreased transpiration-driven mass flow was not a key factor affecting [N] in plants grown in hydroponic culture, in which N could reach the root surface more easily than in soil culture (Conroy 1992; Vessey et al. 1990). It has also been suggested that the increase in N loss is responsible for reducing [N] in plants by eCO₂ (Pang et al. 2006). However, we found little difference between the ratios of the N accumulation and the N consumption from the

nutrient solution per plant in two [CO₂] conditions (Fig. S3), which means the N loss was almost the same under different [CO₂] levels in this study.

In the present work, we aimed to assess the contribution of the short-term fluctuation of [N] in the nutrient solution on [N] in cucumber plants. In low N supply, eCO₂ decreased the Tr per plant by 24.2% (n.s.) (Fig. 2), but slightly increased the N consumption per unit water consumption (Fig. 7), which resulted in a much lower [N] in the nutrient solution (less than 4 mM) (Fig. 3). Although the increase of 24.1% in N consumption and the decrease of 7.9% in water consumption in the whole experimental period were not significant (Fig. 5), it also gave a piece of evidence that eCO₂ significantly decreased [N] in the low N nutrient solution, which aggravated the [N] decrease in plants. In high N supply, eCO₂ increased the Tr per plant by 39.7% (n.s.) (Fig. 2) and increased the N consumption per unit water consumption (Fig. 7), so [N] in the nutrient solution was also lower under eCO₂ than that under aCO₂. However, the final [N] in the nutrient solution was more than 7 mM (Fig. 3), which could still meet the requirements of plants and ensure the regular [N] in plants (Dai et al. 2011). In addition, we found that an increase of 72.9% in N consumption kept pace with an increase of 84.3% in total DW by eCO₂ in the whole experimental period (Fig. 5). It confirms that high N supply could maintain the adequate [N] in the nutrient solution and compensate for the decrease of [N] in plants by dilution effect. Additionally, when N supply was elevated from 7 to 21 mM, the N consumption was respectively increased by 106.9% and 188.1% under eCO₂ and aCO₂ (Fig. 5), which was more than the increase in total DW (71.6% and 126.6%). Conceivably, [N] in plants was significantly increased with the N supply increasing regardless of [CO₂].

Phosphorus concentrations in plants

According to a meta-analysis with 2024 records from 112 published articles, an average decrease of 4.0% in [P] in plants by eCO₂ was found, which was much less than the decrease of [N] (12.7%) (Deng et al. 2015). In a similar meta-analysis, under eCO₂, the leaf C:P ratios in 19 out of 32 species studied were significantly increased, the ratios in five species were significantly decreased, and the ratios in eight species were not significantly affected (Sardans et al. 2012). The uncertainty in the feedbacks of [P] change in plants on eCO₂ mainly

stems from the competition between the dilution effect and the increased metabolic demand for P (Conroy 1992; Gifford et al. 2000). In the present work, besides the dilution effect and the P demand, the effects of the short-term fluctuation of [P] in the nutrient solution on [P] in plants were also evaluated.

In low N supply, eCO₂ increased P requirement for the phosphorylated photosynthetic intermediates and the intercellular transport (Gifford et al. 2000; Jin et al. 2015). Meanwhile, the Tr per plant was slightly decreased by eCO₂ (Fig. 2), so we found a significant increase of the P consumption per unit water consumption under eCO₂ (Fig. 7). Accordingly, [P] in the residual nutrient solution was significantly lower under eCO₂ than that under aCO₂ (Fig. 4) but was still above the 0.6 mM level and sufficient for the cucumber growth (Halbert-Howard et al. 2020; Neocleous and Savvas 2015). Under eCO₂ condition, P consumption and total DW increments were 60.4% and 39.6% (n.s.), respectively (Figs. 1 and 5). It indicated that the increase in P requirement by eCO₂ was more extensive than that in DW accumulation, which resulted in an increase of [P] in stems and fruits of cucumber plants (Fig. 6). In high N supply, eCO₂ increased both Tr per plant and P requirement, which made a synchronous increase in water and P uptakes (Fig. 7). The total water and P consumptions were increased by 27.6% and 27.2% (Fig. 5), respectively, leading to an undifferentiated [P] in the residual nutrient solution at the later growth stage (Fig. 4). Therefore, the decrease or the trend of decrease in [P] in cucumber plants was mainly caused by the increase of total DW accumulation (+84.3%), which was dramatically promoted by eCO₂ coupled with high N supply (Jarrell and Beverly 1981).

Similar to eCO₂, increasing the N supply could also improve the DW accumulation and cause a dilution effect on other mineral element concentrations in plants when the supplies of other mineral elements were not increased accordingly (Halpern et al. 2019; Lenka et al. 2019; Papadopoulos 1986). In the present study, [P] in cucumber plants was significantly decreased with the N supply increasing only under eCO₂ conditions, which indicated other factors rather than dilution effect involved in the changes of [P] in plants. Under aCO₂ conditions, we found a greater P consumption per unit water consumption in high N supply than that in low N supply (Fig. 7), which may be due to the improved root growth and higher root activity in high N supply (Leonce and Miller 1966). Therefore, the increment of

P consumption was 73.7% when the N supply was elevated from N1 to N3, which was equivalent to the increment of total DW (+71.6%) (Figs. 1 and 5). Thus, [P] in cucumber plants stably maintained in different N supply levels (Fig. 6). Under eCO₂ conditions, both Tr per plant and P requirement were increased with the N supply increasing, so the P consumption per unit water consumption has little difference (Fig. 7). Therefore, the significant decrease of [P] in cucumber plants with the N supply increasing was mainly caused by the larger increment of total DW (+126.6%) than that of P consumption (+37.8%) (Figs. 1 and 5) (Halpern et al. 2019; Jarrell and Beverly 1981).

Potassium concentrations in plants

The current study showed that [K] in cucumber plants was 16.3% lower in high N supply under eCO₂ than that under aCO₂ condition (Fig. 6), which was larger than the average [K] decrease of 10% by eCO₂ in previous reviews (Loladze 2002; McGrath and Lobell 2013). It has been frequently suggested that the decrease of [K] in plants by eCO₂ was mainly caused by the biomass dilution (Fernando et al. 2014; Halpern et al. 2019; Zhu et al. 2016). However, the differences in [K] in plants between two [CO₂] conditions in low N supply were slight (Fig. 6), so there must be other factors in addition to dilution effect involved in the decrease of [K] in plants by eCO₂. In this work, we focused on the short-term fluctuation of [K] in the nutrient solution.

In low N supply, eCO₂ had little effect on the Tr per plant (Fig. 2). Although the K consumption per unit water consumption was increased by eCO₂ (Fig. 7), the differences in [K] in the residual nutrient solution between two [CO₂] conditions were not significant, and the final [K] was more than 5 mM. The adequate and stable [K] in the nutrient solution afforded the increase of K consumption by (+47.6%, n.s.) (Fig. 5), which was parallel to the increase of total DW accumulation (+39.6%, n.s.). Thus, [K] in all parts of cucumber plants did not change by eCO₂ (Fig. 6). In high N supply, the Tr per plant had an increasing trend by eCO₂ (Fig. 2), and the K consumption per unit water consumption was moderately increased (Fig. 7). Therefore, increment in K consumption (+65.1%) in the whole experimental period was larger than that in total water consumption (+27.6%) (Fig. 5), resulting in a steep decline in [K] in the residual nutrient solution, especially at the later growth stage (Fig. 4). The final [K] in the nutrient

solution was about 3 mM, only a half of the initial value, which severely aggravated the [K] decline in plants under eCO₂ (Halbert-Howard et al. 2020; Neocleous and Savvas 2015; Savvas et al. 2014). Moreover, the increase in total DW accumulation by eCO₂ (84.3%) outstripped that in K consumption (+65.1%), which indicated that the decrease of [K] in all parts of cucumber plants by eCO₂ was inevitable (Fig. 6).

Meanwhile, N supply also exerted a significant impact on [K] in cucumber plants. Under aCO₂ conditions, although increasing the N supply promoted the K consumption per unit water consumption and decreased [K] in the residual nutrient solution (Figs. 4 and 7), [K] in the nutrient solution (more than 5 mM) could afford the cucumber growth. Therefore, the decrease of [K] in roots and increase of [K] in leaves with N supply increasing were mainly caused by the synchronous uptake of K⁺ with NO₃⁻ (Li et al. 2020; Zhang et al. 2010). Previous studies showed that K⁺ was always transported in xylem sap accompanied by NO₃⁻ from roots to shoots to maintain the balance between anions and cations (Blevins et al. 1978; Triplett et al. 1980). Under eCO₂ conditions, the uptake of K⁺ was significantly increased with increasing N supply due to the synchronous uptake of K⁺ with NO₃⁻. It was evidenced by the synchronized increment of consumptions of K (+122.7%) and N (+188.1%) when the N supply was increased from 7 to 21 mM, whereas the corresponding increment of water consumption was only 75.9% (Fig. 5). Therefore, increasing the N supply enormously decreased [K] in the residual nutrient solution, especially at the later growth stage, and the decrease of [K] fluctuated from 25.1 to 41.2% (Fig. 4). The deficiency of K in the nutrient solution severely inhibited the further uptake of K⁺, leading a significant decrease of [K] in roots and fruits of cucumber plants when the N supply was increased from 7 to 21 mM (Fig. 6).

Conclusions

In the present study, we found eCO₂ inhibited the Tr per plant increment but improved the N consumption in low N treatment. Therefore, [N] in the nutrient solution in the closed hydroponic system decreased significantly too far below the demand of cucumber plants, which imposed an additional [N] decrease in plants besides the dilution effect by eCO₂. [P] in the nutrient solution slightly fluctuated during one round of nutrient solution

treatment and was always abundant for the cucumber growth regardless of the $[\text{CO}_2]$ and N supply levels, so the change of [P] in the cucumber plants was mainly attributed to the dilution effect and the demand change. [K] in the nutrient solution decreased significantly in moderate and high N treatments due to the synchronous uptake of K^+ with NO_3^- , which would exacerbate the [K] decrease in plants under $e\text{CO}_2$.

Back to our hypothesis posed at the beginning, it is safe to state that the asynchronous change in the uptake of N, K, and water under $e\text{CO}_2$ compared with that under $a\text{CO}_2$ results in a short-term fluctuation of [N] and [K] in the nutrient solution in the commonly used closed hydroponic systems. Moreover, this fluctuation of nutrient concentrations due to the defect of experimental set-ups could lead to an appreciable decline of [N] and [K] in plants, which may let us exaggerate the negative impact of $e\text{CO}_2$ itself. It is recommended to renew the nutrient solution as frequently as possible, to eliminate the influence of nutrient fluctuation in solutions on the plant nutritional status and distinguish the real effects of $e\text{CO}_2$. Savvas and Adamidis (1999), and Zheng et al. (2010) also suggested using bigger growth containers or continuously flowing nutrient solution with constant elemental concentrations controlled by automated systems. Furthermore, a practical implication is that the improved nutrient solution formulations based on the nutrient requirements of plants under $e\text{CO}_2$ condition are urgently needed for the greenhouse vegetable production with $e\text{CO}_2$ fertilization or for the field crop production in future climate scenarios. An appropriate increase in N and K supply is recommended under $e\text{CO}_2$ condition in the present study. Thus, the sustainable crop production avoiding photosynthetic acclimation and utilizing the benefits of $e\text{CO}_2$ could be achieved in future climate change scenarios (Gruda et al. 2019a, b).

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