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Nitrogen management under increased atmospheric CO₂ concentration in cucumber (*Cucumis sativus* L.): ameliorating environmental impacts of fertilization

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In the last years, the atmospheric CO₂ concentration has increased significantly, and this increase can cause changes in various physiological and biochemical processes of plants. However, the response of plants to elevated CO₂ concentration (e[CO₂]) will be different depending on the nitrogen form available and the plant species. Therefore, hydroponic trials on cucumber plants, with two CO₂ concentrations (400 and 1000 ppm) and two nitrogen sources (NO₃⁻/NH₄⁺; 100/0 and 90/10), were conducted. Physiological parameters—such as gas exchange, GS, GOGAT and GDH activities, cation composition, soluble sugar and starch content—were measured. The results showed that when plants were grown with NH₄⁺ and e[CO₂], parameters such as photosynthesis rate (A_{CO₂}), instantaneous water use efficiency (WUEi), the content of NH₄⁺, Ca²⁺ and Mg²⁺, and the concentration of starch, were higher than in control plants (irrigated with nitrate as sole nitrogen source and ambient CO₂ concentration (a[CO₂])). Furthermore, an improvement in N assimilation was observed when the GS/GOGAT pathway was enhanced under these conditions (NH₄⁺ and e[CO₂]). Thus, our results contribute to the reduction of the negative environmental impacts of the use of nitrogen fertilizers on this crop, both by reducing nitrogen leakage (eutrophication) and greenhouse gas emissions.

The atmospheric carbon dioxide (CO₂) concentration is increasing at a faster rate and is projected to reach nearly 1000 μmol mol⁻¹ by the end of 2100¹. Since the current CO₂ concentration (413 ppm) (NOAA 2020) is still a limiting factor for plant growth, and the optimal concentration is considered between 800–1000 ppm², this increase in CO₂ concentration could favor the photosynthetic rate and stimulate plant growth and development. However, the response of plants to e[CO₂] varies depending on other environmental factors³, such as the nitrogen form (NO₃⁻ or NH₄⁺) available⁴. Authors such as Rubio-Asensio and Bloom⁴ reported that plants irrigated with NH₄⁺ showed a more positive response to e[CO₂], than those irrigated with NO₃⁻, as e[CO₂] inhibited the assimilation of NO₃⁻ in the shoots of C3 plants.

Both N forms (NO₃⁻ and NH₄⁺) share the same metabolic pathway, called as glutamate pathway, in which enzymes such as glutamate synthase (GOGAT) and glutamine synthase (GS) are involved; hence, this pathway is also identified as the GS/GOGAT cycle. GS is responsible for assimilating NH₄⁺ as amino acids (glutamine), and GOGAT transforms this glutamine to glutamate⁵. Furthermore, glutamate dehydrogenase (GDH) catalyzes the transformation of α-ketoglutaric acid and NH₃ into glutamate through the glutamate pathway. Consequently, GOGAT, GS and GDH are considered the principal enzymes of N metabolism in higher plants⁵. As the response of plants to an increase in CO₂ concentration will be different depending on the source of N supplied and the specie considered, it will be very important to better understand the plant's preference for different N forms under e[CO₂], as this knowledge can be used to guide farmers towards the more efficient use of fertilizers. Therefore, because of the high energy cost of production of N fertilizers, and the environmental impact of the nitrate that is not taken up by plants⁶, growers must reduce the N losses and pollution associated to fertilization by using the

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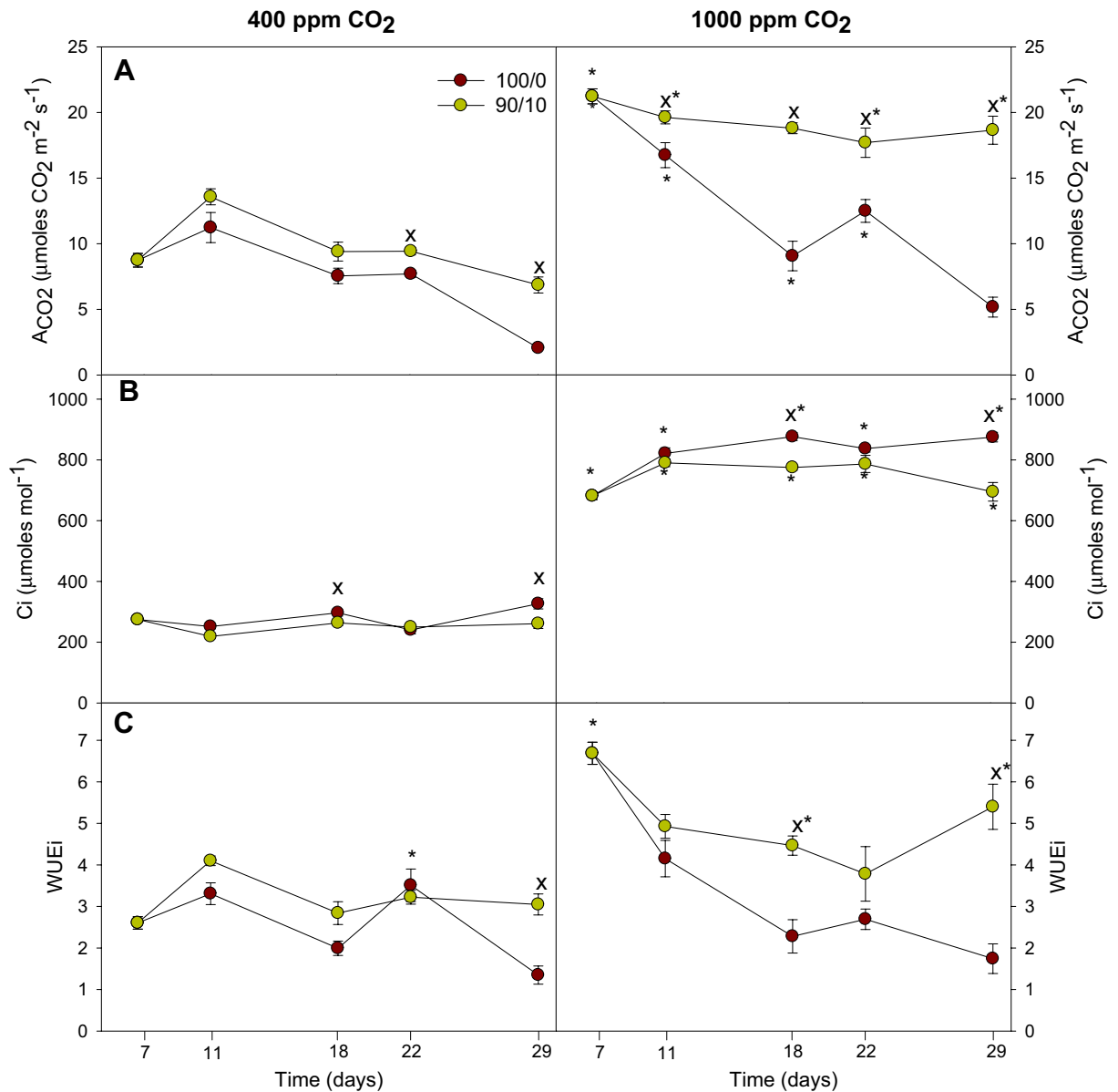


Figure 1. Effect of N forms (100% NO_3^- and 90% $\text{NO}_3^-/10\%$ NH_4^+ combined) under an $e\text{CO}_2$ on cucumber plants: **(A)** net photosynthesis rate (A_{CO_2}); **(B)** internal CO_2 concentration (C_i); and **(C)** instantaneous water-use efficiency (WUEi). Data are means \pm SE of six plants. * denotes significant differences ($P < 0.05$) between plants in different CO_2 treatments, with the same nitrogen treatment; X denotes significant differences ($P < 0.05$) between nitrogen treatments for the same CO_2 treatment.

appropriate ratio of inorganic nitrate under future climate scenarios. The nitrogen fertilization of crops is one of main causes of environmental contamination worldwide, through nitrate leaching and as a net contributor to greenhouse gas emission^{7,8}. Cucumber (*Cucumis sativus* L.) is one of the most-cultivated vegetables in the world due to its economic and nutritional benefits^{5,9}. However, although many cucumber growth parameters have been studied, such as photosynthesis¹⁰, nitrogen metabolism¹¹, fruit quality¹², or water use efficiency¹³, the combined effects of CO_2 and different N forms (NO_3^- and NH_4^+) have not been studied. Therefore, this study is the first attempt at understanding how N forms and $e[\text{CO}_2]$ interact in cucumber plants in a climate change scenario. To stimulate the physiological mechanisms affected by these two factors (N forms and $e[\text{CO}_2]$), cucumber plant seedlings were exposed to different N inputs and $e[\text{CO}_2]$ in a controlled environment. The responses of plants were assessed by measuring the net CO_2 assimilation, internal CO_2 concentration, instantaneous water use efficiency, cation concentration, nitrogen-metabolizing enzymes, starch, and soluble sugars.

Results

Gas exchange. The data showed that the treatment with NH_4^+ (90/10) increased the A_{CO_2} at both CO_2 concentrations (Fig. 1A). The A_{CO_2} throughout the experiment slightly decreased at both CO_2 concentrations. Curiously, the decrease was more pronounced when the plants were irrigated with the 100/0 treatment at both CO_2

[CO ₂]	Nitrogen form	NH ₄ ⁺ (mmol Kg ⁻¹)	Ca ²⁺ (mmol Kg ⁻¹)	K ⁺ (mmol Kg ⁻¹)	Mg ²⁺ (mmol Kg ⁻¹)
400	100/0	5.68 ± 0.08 ^b	6.21 ± 0.38 ^d	118.5 ± 9.8 ^a	19.39 ± 2.46 ^c
	90/10	5.92 ± 0.04 ^b	13.70 ± 0.40 ^b	120.4 ± 4.4 ^a	32.44 ± 2.38 ^{ab}
1000	100/0	3.83 ± 0.30 ^c	9.41 ± 1.13 ^c	133.2 ± 5.1 ^a	25.53 ± 3.47 ^{bc}
	90/10	7.00 ± 0.27 ^a	17.98 ± 0.61 ^a	123.7 ± 2.9 ^a	34.26 ± 1.63 ^a
ANOVA^b					
CO ₂		ns	***	ns	ns
NF ^c		***	***	ns	***
CO ₂ × NF		***	ns	ns	ns

Table 1. Effect of N forms (100% NO₃⁻ and 90% NO₃⁻/10% NH₄⁺ combined) under an elevated CO₂ concentration on the cation composition of leaves of cucumber. ^aDifferent letters within a column indicate significant ($P \leq 0.05$) differences between treatments. ^bAnalysis of variance: ns, not significant. ^cNitrogen form. * $P \leq 0.05$. ** $P \leq 0.005$. *** $P \leq 0.001$.

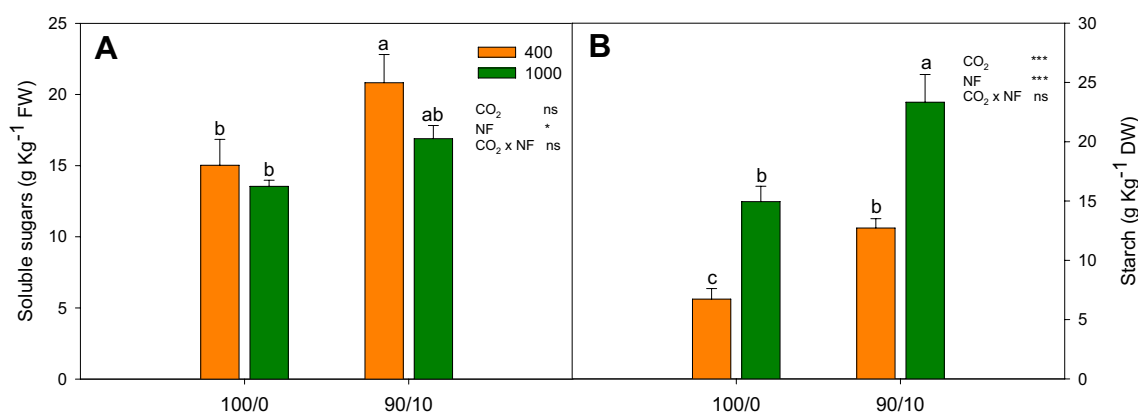


Figure 2. Effect of N forms (100% NO₃⁻ and 90% NO₃⁻/10% NH₄⁺ combined) under an elevated CO₂ concentration on leaves of cucumber: (A) soluble sugars; and (B) starch. Data are means ± SE of six plants. Different letters indicate significant ($P \leq 0.05$) differences between treatments.

concentrations, but mainly under e[CO₂]. This decrease was from 21.2 μmoles CO₂ m⁻² s⁻¹ to 5.2 μmoles CO₂ m⁻² s⁻¹ in plants irrigated with 100/0 under e[CO₂]. In contrast, the decrease in plants irrigated with the 100/0 treatment under a[CO₂] was only from 8.7 μmoles CO₂ m⁻² s⁻¹ on 7 DAT, to 2.1 μmoles CO₂ m⁻² s⁻¹ on 29 DAT. On the other hand, exposure to e[CO₂] markedly increased A_{CO2} in both N treatments (Fig. 1A). Similarly, the Ci increased by CO₂ enrichment (Fig. 1B). However, this parameter obtained higher values in plants irrigated with the 100/0 treatment at both CO₂ concentrations, on 18 DAT and 29 DAT. During the rest of the experiment, no significant differences were observed between the irrigation treatments.

With regard to the WUEi, the levels were higher in plants irrigated with the 90/10 treatment under e[CO₂] than under a[CO₂] (Fig. 1C). The 90/10 treatment also caused a slight increase of this parameter with respect to plants irrigated with NO₃⁻ as the sole N source, but with differences significant only on 29 DAT at a[CO₂] (56%) and on 18 and 29 DAT at e[CO₂] (49% and 68%, respectively).

Mineral concentration. The mineral composition was affected by CO₂ and N form (Table 1). The leaf NH₄⁺ and Ca²⁺ concentrations were higher at e[CO₂] than at a[CO₂] in both N treatments, with the only exception found in the NH₄⁺ concentration of plants irrigated with the 100/0 treatment, which was reduced (33%). Also, the addition of NH₄⁺ to the irrigation solution caused an increase in the concentration of Ca²⁺ and Mg²⁺ at both CO₂ concentrations. But in the case of NH₄⁺ concentration, this increase was only observed under e[CO₂] (15%). In contrast, leaf K⁺ concentration was not affected by neither CO₂ nor N form (Table 1).

Soluble sugars and starch. The soluble sugars and starch contents were affected by N form (Fig. 2A and B). The sugar content increased mainly under the 90/10 treatment at a[CO₂] (from 15 g Kg⁻¹ FW to 21 g Kg⁻¹ FW), while at e[CO₂], this increase was not significant (Fig. 2A). The starch content rose under both CO₂ concentrations by the combined application of NO₃⁻ and NH₄⁺ (from 7 g Kg⁻¹ DW to 13 g Kg⁻¹ DW under a[CO₂] and from 15 g Kg⁻¹ DW to 23 g Kg⁻¹ DW under e[CO₂]) (Fig. 2B). On the other hand, the e[CO₂] provoked an increase in the starch content, from 7 g Kg⁻¹ DW to 15 g Kg⁻¹ DW in plants irrigated with 100/0 and from 13 g Kg⁻¹ DW to 23 g Kg⁻¹ DW, in plants irrigated with 90/10 (Fig. 2B).

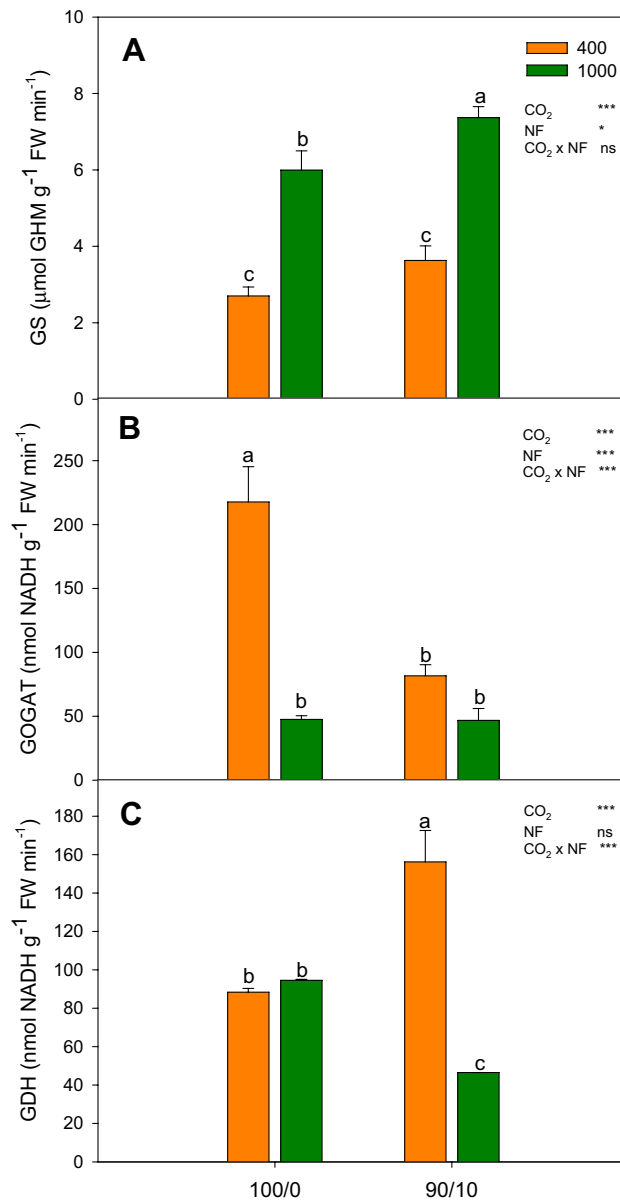


Figure 3. Effect of N forms (100% NO₃⁻ and 90% NO₃⁻/10% NH₄⁺ combined) under an elevated CO₂ concentration on leaves of cucumber: (A) glutamine synthetase activity (GS); (B) glutamate synthase activity (GOGAT); and (C) glutamate dehydrogenase activity (GDH). Data are means ± SE of six plants. Data are means ± SE of six plants. Different letters indicate significant ($P \leq 0.05$) differences between treatments.

Nitrogen-metabolizing enzymes. The behaviors of these 3 enzymes (GS, GOGAT, and GDH) against the N source were dependent on the environmental conditions in which the plants were grown (Fig. 3). Plants grown under a[CO₂] and irrigated with the 90/10 treatment, suffered a significant reduction in GOGAT activity (62%), and an increase in GDH activity (43%) (Fig. 3B and C). On the contrary, when plants were grown under e[CO₂], the treatment with a mixture of N forms caused a slight increase in GS activity (19%) and a reduction in GDH activity (51%) (Fig. 3A and C).

Discussion

The effects on plants of the N form(s) used for irrigation depend on several factors such as environmental conditions (temperature, light intensity, atmospheric CO₂ concentration, N concentration, average pH, and K supply), the proportions in which they are supplied (NO₃⁻/NH₄⁺), the plant species, and even on the variety. Therefore, studies carried out by various authors about the use of different N sources have shown different results^{14–16}.

Our data showed that the combination of different N forms (NO₃⁻/NH₄⁺) and e[CO₂] provoked a significant increase in the gas exchange parameters. In our previous studies, we observed a similar behavior in pepper plants exposed to similar conditions^{17,18}. Something similar was reported by authors such as Cruz et al.¹⁹ and Bloom et al.²⁰, who observed that plants (cassava and wheat plants, respectively) exposed to e[CO₂] and NH₄⁺-based

nutrition showed a greater stimulation of photosynthesis than those irrigated with NO_3^- alone. These results indicate that adding NH_4^+ to the nutrient solution increases the photosynthetic capacity of plants grown under $e[\text{CO}_2]$. This could be due to the energy cost involved in assimilating NO_3^- versus NH_4^+ : the reduction of NO_3^- to NH_4^+ implies the consumption of approximately 10 ATP molecules, while in the conversion of NH_4^+ to glutamate, the consumption is reduced to only approximately 2 ATP⁴. Therefore, plants fed with NO_3^- as the N source must efficiently divide and distribute the reducer generated in photosynthesis to cover the additional assimilation demands of NO_3^- assimilation⁴. In the case of WUEi, Cruz et al.¹⁹ just as in the present study, observed the highest WUEi values under $e[\text{CO}_2]$ and NH_4^+ nutrition. However, authors such as Torralbo et al.²¹ found the opposite effect on photosynthesis in durum wheat, but a similar behavior in WUEi.

Under $e[\text{CO}_2]$ conditions, the foliar NH_4^+ concentration results were different depending on the N source supplied to the roots. This is in agreement with the results found by Rubio-Asensio and Bloom⁴, who observed that CO_2 enrichment inhibited NO_3^- assimilation in plants grown with NO_3^- nutrition, but did not affect those grown with NH_4^+ nutrition. This would explain the lower NH_4^+ concentration observed in plants treated with 100/0 under $e[\text{CO}_2]$. In these plants, as NH_4^+ is not provided in the nutrient solution, the foliar NH_4^+ concentration observed came from the assimilation of NO_3^- , which changes from NO_3^- to nitrite (NO_2^-) and next, to NH_4^+ ²². This suggests that future increases in atmospheric CO_2 concentrations may compromise the productivity of some plants if we do not change the fertilization strategies.

Curiously, other nutrients such as Ca^{2+} and Mg^{2+} increased in concentration with the 90/10 treatments at both CO_2 concentrations. However, authors such as Boschiero et al.²³ reported contrary effects in sugarcane plants, which showed a reduction in the leaf nutrients with the increase in the $\text{NO}_3^-/\text{NH}_4^+$ ratio. The fact that the 90/10 treatment produced an increase in the foliar Ca^{2+} concentration, despite the antagonistic effect that exists between these two elements¹⁸, could indicate that at a low concentration of NH_4^+ , this antagonistic effect does not occur for cucumber plants. A significant positive correlation was found between foliar Ca^{2+} content and WUEi ($R^2=0.6708$, data not shown). It is known that Ca^{2+} plays an important role in multiple photosynthetic pathways, it can both interfere with gas exchange by regulating stomatal movement, and can directly or indirectly regulate the activity of enzymes involved in photosynthesis. Authors such as Brestic et al.²⁴ observed that Ca^{2+} improved Rubisco activity, and the higher activity seemed to be associated with a higher photosynthetic rate.

Concerning the values of K^+ , the finding that no differences were observed in foliar K^+ concentration between treatments, could be another indication that the NH_4^+ concentration provided was not toxic for cucumber plants, as K^+ deficiencies have been observed in toxic concentrations due to competition in absorption between K^+ and NH_4^+ ²³. Regarding the CO_2 effect on the foliar nutrient content, curiously the cucumber plants responded in the opposite manner to the pepper plants under similar growth conditions¹⁸, which reinforces the idea of significant species-dependent-response.

Authors such as Teng et al.²⁵, Markelz et al.²⁶ and Rubio-Asensio and Bloom⁴, observed that $e[\text{CO}_2]$ provoked an increase in the photosynthesis rate of *Arabidopsis*, which was related with a rise in starch and the total non-structural carbohydrates. In our experiment, something similar was observed with the starch, but no changes were observed in the soluble sugars. This could be due to the increase in starch or other carbohydrates storage polymers being greater than the increase in sugar concentrations, but the extent of the changes vary considerably between species²⁷. Also, it is known that the N supplied can have an influence on the accumulation of starch and soluble sugars under $e[\text{CO}_2]$ ²⁷. In our results, a relationship was once more observed between the increase caused by NH_4^+ in photosynthesis and the higher leaf starch content observed.

Regarding the behavior observed in the metabolic enzymes measured, our data showed that the treatment with NH_4^+ under an atmosphere enriched with CO_2 provoked an increase in the GS activity and a reduction in the GDH activity, which indicate that the combination of the 90/10 treatment and $e[\text{CO}_2]$ may promote the GS/GOGAT pathway of N metabolism. This increase in the GS/GOGAT pathway could be partly responsible for a higher photosynthesis rate, as it would favor N assimilation⁵. On the contrary, the treatment with NH_4^+ under $a[\text{CO}_2]$ conditions resulted in a higher GDH activity and lesser GOGAT activity than the 100/0 treatment. These results suggest that GDH played a decisive role when the GS/GOGAT pathway was restricted. Authors such as Ma et al.⁵ and Torralbo et al.²¹ consider that the role of GDH in N metabolism becomes more important when plants are subjected to stress, specifically NH_4^+ toxicity. GDH removes excess NH_4^+ and thus reduces its toxic effect.

To achieve a better understanding of the trends and relationships among all the studied parameters in relation to the N supply, a PCA was applied to the results. The results of the PCA are presented in Table 2 and presents a clearer distinction of the effects of nitrogen form and CO_2 concentration. The first two principal components (PCs) accounted for 71.38% of the total variance, attributing 42.26% to PC1 and 29.13% to PC2 (Table 2). Most of the variables examined were positively correlated with PC1, and only two variables were negatively correlated with PC1. The variables with the highest positive correlation coefficients were Ca^{2+} (0.925) and WUEi (0.877), and others with a high correlation were soluble sugars (0.685), Mg^{2+} (0.559), A_{CO_2} (0.772) and NH_4^+ (0.780). PC1 was negatively correlated with GDH (-0.402) and K^+ (0.762), allowed for a clear separation of plants irrigated with NH_4^+ in the nutrient solution, and suggested that plant growth with the 90/10 treatment was characterized by a higher Ca^{2+} (Table 1), and higher WUEi (Fig. 1C). PC2 was positively correlated with GS (0.871), starch (0.690) and Ci (0.944), and was negatively correlated with GOGAT activity (-0.669). The PC2 clearly separated plants grown under $e[\text{CO}_2]$, characterized by a higher GS activity (Fig. 2A), and higher Ci (Fig. 1B).

The data obtained in this experiment highlight the complexity and importance of using the correct type of nitrogen fertilization in the plant irrigation solution to face the environmental changes that are currently taking place (increase in CO_2). We have demonstrated that physiological parameters such as the A_{CO_2} and WUEi can be improved in cucumber plants with the addition of NH_4^+ in low amounts in the nutrient solution under a CO_2 -enriched atmosphere. Also, under these conditions (NH_4^+ and $e[\text{CO}_2]$), the GS/GOGAT cycle is promoted, which favors the assimilation of N, and the increase in the concentrations of other nutrients such as NH_4^+ , Ca^{2+} , and Mg^{2+} , and the starch content.

Principal components	1	2
Eigenvalues	5.493	3.786
Proportion of variation	42.256	29.125
Accumulated proportion of variation	42.256	71.381
Characters	Eigenvectors	
A _{CO2}	0.772	
WUE _i	0.877	
GDH activity	-0.402	
NH ₄ ⁺	0.780	
Ca ²⁺	0.925	
K ⁺	-0.762	
Mg ²⁺	0.925	
Soluble sugars	0.685	
C _i		0.944
GS activity		0.871
GOGAT activity		-0.669
Starch		0.690

Table 2. Eigenvalues, proportion of variation, accumulated proportion of variation, and eigenvectors associated with the two axes of the PCA.

Consequently, this study reveals the strong interaction between the N form supplied and e[CO₂], in terms of N assimilation, and therefore, of a better performance of the photosynthetic apparatus.

Material and methods

Plant material, growth conditions and treatments. Cucumber (*Cucumis sativus* L.), cv. Ashley seeds (Semillas Batlle, S.A., Barcelona, Spain) were germinated on a mixture of peat and perlite (3:1). Seedlings with two true leaves stages were selected for uniformity after the 12 days, and transplanted to 8-L black containers filled with coconut coir fiber (Pelemix, Alhama de Murcia, Murcia, Spain). Each container was rinsed with 1 L of water after transplanting. Irrigation was supplied by self-compensating drippers (2 l h⁻¹), and fresh nutrient solution was applied with a minimum of 35% drainage.

The plant growth responses to different nitrogen forms and e[CO₂] were determined in an experiment carried out in a climate chamber designed by our department specifically for plant research proposals²⁸, with fully-controlled environmental conditions: 30% relative humidity, 16/8 h day/night photoperiod, an air temperature ranging from 28 to 20 °C, and a photosynthetically-active radiation (PAR) of 250 μmol m⁻² s⁻¹ provided by a combination of fluorescent lamps (TL-D Master reflex 830 and 840, Koninklijke Philips Electronics N.V., the Netherlands) and high-pressure sodium lamps (Son-T Agro, Philips). During the first seven days after transplanting (7 DAT), the plants were irrigated with Hoagland's solution (control), and then, the plants were irrigated with Hoagland's solutions that differed in their NO₃⁻/NH₄⁺ ratios (in concentration percentages, 100/0 or 90/10) for twenty-two days.

The experiment lasted twenty-nine days and was carried out at standard CO₂ (400 μmol mol⁻¹ CO₂) (a[CO₂]), and elevated CO₂ (1000 μmol mol⁻¹ CO₂) (e[CO₂]) concentrations, with nine plants per treatment. Thus, four treatments were studied, corresponding to two nutrient solutions and two ambient CO₂ concentrations.

Statistical analysis. Data were statistically analyzed using the SPSS 13.0 software package (IBM SPSS Statistics 25.0, Armonk, NY, USA), with an ANOVA and Duncan's multiple range test ($P \leq 0.05$) using the treatments as a statistical variable to determine significant differences between means.

Gas exchange. The gas exchange measurements were performed just before starting the nitrogen treatments (7 DAT), and throughout the experiment (11, 18, 22, and 29 DAT). A CIRAS-2 (PP system, Amesbury, MA, USA) with a PLC6 (U) Automatic Universal Leaf Cuvette, was used to measure the net CO₂ assimilation (A_{CO2}), internal CO₂ concentration (C_i) and instantaneous water-use efficiency (WUE_i, A_{CO2}/E). The measurements were conducted on the youngest fully-expanded leaf from each plant. The cuvette provided light (LED) with a photon flux of 1300 μmol m⁻² s⁻¹, 400 or 1000 μmol mol⁻¹ CO₂, 70% relative humidity, and a leaf temperature of 26 °C.

Ion concentrations. The NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ ions were extracted from ground leaf lyophilized (1 g) with bi-distilled water, and their concentrations were determined in an ion chromatograph (METROHM 861 Advanced Compact IC; METROHM 838 Advanced Sampler); the column used was a METROHM Metrosep C1 125/4.6 mm.

Starch and soluble sugars. Soluble sugars were extracted by incubating 30–40 mg of lyophilized leaf tissue twice in 5 mL of 60% ethanol, 30 min each time, at 35 °C. The extract was centrifuged at 3500×g for 10 min at 20 °C, and the two supernatants were combined. Chloroform (5 mL) was added and the mixture shaken before centrifugation at 2700×g for 10 min at 20 °C. The sample was diluted fourfold with absolute ethanol to produce an extract in 80% ethanol for the measurement of soluble sugars according to Buysse and Merckx²⁹. The residual material from the extraction with 60% ethanol was hydrolyzed with 3% HCl for 3 h at 125 °C, and the soluble sugars released were measured as an estimate of the starch content³⁰.

Nitrogen-metabolizing enzymes extraction and assay. Fresh leaf tissue samples were frozen with liquid nitrogen and stored at –20 °C until analysis. Between 0.5 g of plant tissue were pulverized under liquid nitrogen with a chilled pestle and mortar and then homogenized with 5 mL ice-cold enzyme extraction buffer containing 50 mM Tris–HCl, pH 8; 1 mM EDTA, 10 mM β-mercaptoethanol, 5 mM dithiothreitol (DTT), 10 mM MgSO₄·7H₂O, 6.6% of PVPP (polyvinylpolypyrrolidone), 1 mM Cysteine, and 0.5 mM phenylmethylsulfonyl (PMSF).

After centrifugation at 17,000×g at 4 °C for 20 min, the supernatant was collected and used for enzyme assays. The activity of NADH-GOGAT (EC 1.4.1.14.) and GDH (EC 1.4.1.2.) were assayed spectrophotometrically according to Groat and Vance³¹ by monitoring the oxidation of NADH at 340 nm. The activity of glutamine synthetase (GS, EC 6.3.1.2) was assayed spectrophotometrically according to the modified method by Setiën et al.³², and the absorbance of γ-glutamyl monohydroxamate (γ-GHM) was measured at 540 nm.

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Author contributions

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Competing interests

The authors declare no competing interests.

Additional information

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