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Short- and long-term effects of elevated CO_2 on the growth and nitrogen content of *Panax japonicus*

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Abstract: *Panax japonicus* (T. Nees) C.A. Mey is a rare plant that is used in Chinese herbal medicine. Two-year-old *Panax japonicus* seedlings were grown in open-top chambers under ambient CO_2 (aCO_2), moderately ($e1CO_2$: 550 ± 15 µmol/mol) or substantially ($e2CO_2$: 750 ± 15 µmol/mol) elevated CO_2 to study their effects on plant growth and nitrogen (N) content. After 27 days, the net photosynthetic rate (P_n) of $e2CO_2$-treated seedlings was significantly higher (20.09%) than that of seedlings grown under aCO_2. However, by day 51, the Pn of $e1CO_2$- and $e2CO_2$-treated seedlings was 12.87% higher and 9.57% lower, respectively, than that of seedlings grown under aCO_2. On day 27, chlorophyll *a* + *b* content, Rubisco activity, soluble protein and starch content of $e1CO_2$- and $e2CO_2$-treated seedlings showed no significant change and significant increase compared to aCO_2-treated. By contrast, at 51 days, chlorophyll *a* content, Rubisco activity, starch and N content of *P. japonicus* was significantly increased by moderately eCO_2 condition but was significantly decreased by substantially eCO_2 condition. The results suggest that exposure to moderately eCO_2 was beneficial for *P. japonicus* growth, whereas under substantially eCO_2, growth were promoted in short-term but inhibited in long-term. Owing to the temporal effect of eCO_2 on *P. japonicus* growth, there was no significant correlation between N content and P_n of *P. japonicus* under eCO_2.

Keywords: elevated CO_2; nitrogen; *Panax japonicus;* photosynthesis; temporal effect

The greenhouse phenomenon, driven by the escalating levels of atmospheric carbon dioxide (CO_2), is now widely acknowledged as a key driver behind the accelerated pace of global climate change (IPCC 2014). Before the industrial revolution, the atmospheric CO_2 concentration was approximately 280 ppm. Since then, levels have been rising, and in the past decade have been increasing at a rate of about 2 ppm per year (Franks et al. 2013), reaching 418 ppm in March 2022 (Ripple et al. 2022). In most cases, if no long-term regulation of photosynthesis occurs, the rate of photosynthesis rises under elevated CO_2 (eCO_2) concentrations (Kimball 2016). However, studies have shown that although the atmospheric CO_2 concentration has been increasing over the past 50 years, the increase in the net photosynthetic rate (P_n) has been much lower than that predicted by the Farquhar photosynthesis model, this is probably because the early experimental results ignored the long-term "CO_2 domestication" effect (Jin et al. 2019). Although studies have suggested that exposure to eCO_2 can increase plant biomass, there is now a large body of evidence to suggest that these increases in plant biomass may be more short-lived than previously anticipated, and that plant responses under future eCO_2 atmospheric conditions may

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be limited by physiological, population, community, and ecosystem-level dynamics (Norby 2021). Therefore, it is important to consider the temporal effect of eCO_2 when studying the effect of eCO_2 concentrations on plant growth. Most plants show reduced photosynthetic efficiency under long-term exposure to eCO_2 (Wang et al. 2021). For example, when *Polygonatum kingianum*was exposed to eCO_2 for a long period of time, the promotion of photosynthesis was not sustained and "CO_2 domestication" occurred (Luo et al. 2023).

Studies have suggested that possible explanations for the observation that nitrogen (N) nutrition is reduced under eCO_2 conditions include N utilisation is hindered (Adavi, Sathee 2021), a growth dilution effect (Deng et al. 2015), reduced N demand and uptake, and N losses (Taub, Wang 2008). N plays an important role in regulating plant growth in response to eCO_2, and the reduction of photosynthetic efficiency caused by long-term eCO_2 can be mitigated by altering the form and amount of N (Wang et al. 2021). Analysing the N nutritional status of plants exposed to eCO_2 is useful to regulate the effects of eCO_2 on plant growth through N regulation.

Herbal medicines are among the oldest alternative and complementary medicines. The impact of climate change, especially eCO_2, on the cultivation of some plants used in Chinese herbal medicines has been studied, including *Pseudostellaria heterophylla* (Ng et al. 2022), *Hypericum perforatum* (Sharma et al. 2020), *Isatis indigotica* (Hao et al. 2013), *Gynostemma pentaphyllum* (Chang et al. 2016), *Gynura bicolor* (Ren et al. 2014), and *Paris polyphylla* (Qiang et al. 2020). *Panax japonicus* (T. Nees) C.A. Mey, a perennial herbaceous plant that belongs to the family Araliaceae, is one of the rare, endangered, and valuable "seven types" of Chinese herbal medicines. Most studies on *P. japonicus* have focused on chemical composition, pharmacological activity, quality evaluation, and metabolic regulation by genetic engineering rather than on cultivation physiology and ecology. In particular, there has been little study of the effects of global climate change, particularly eCO_2, on *P. japonicus*. To better understand the impact of eCO_2 on *P. japonicus* growth, and to determine a theoretical basis for *P. japonicus* cultivation under climate change conditions, we analysed: (*i*) the temporal effects of moderately eCO_2 and substantially eCO_2 conditions on the photosynthesis, growth, and N content of *P. japonicus*; (*ii*) the relationship between N content and photosynthesis.

MATERIAL AND METHODS

Materials

We used the underground rhizome of 2-year-old *P. japonicus* plants cultivated from the seed of the same mother plant. In November 2022, underground rhizomes with consistent growth were transplanted into plastic pots (diameter 21 cm; height 12 cm) containing 2.0 kg of air-dried potting soil comprising brown loam and nutrient soil (1 : 1), with one rhizome per pot.

Experimental design

We used an open-top air chamber method to simulate eCO_2 conditions. The experiment involved three CO_2 concentration levels: the ambient level (aCO_2) and two elevated levels ($e1CO_2$ and $e2CO_2$). The aCO_2 gas chamber was vented to natural air with a CO_2 concentration of 350 ± 80 μmol/mol. The $e1CO_2$ and $e2CO_2$ gas chambers were vented to CO_2 gas with a controlled CO_2 concentration of 550 ± 15 μmol/mol and 750 ± 15 μmol/mol, respectively. Three replicates were set up for each treatment, with 15 seedlings per replicate (i.e. 45 seedlings per treatment). The soil was watered as needed over the course of the experiment to keep the soil moist; any weeds that germinated were removed. The eCO_2 treatments were initiated on May 11, 2023. The P_n was measured on sunny days, i.e. on day 0, 17, 27, 39, 51, 70, 84, and 95 of the CO_2 treatment. Research indicators were measured on days 27 and 51 of the CO_2 treatment.

Parameter measurement

Determination of net photosynthetic rate (P_n). The P_n of leaves was determined using a Li-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). Three plants in each treatment were selected. Three fully expanded functional leaves from the middle and upper part of each plant were selected and labelled. The P_n of these leaves was measured on sunny days from 9 a.m. to 11 a.m. (Zhang, Zhang 2022).

Determination of photosynthetic pigment content, Rubisco activity, and soluble sugar, starch, soluble protein, and free amino acid content levels. The upper functional leaves (0.2 g) of five seedlings in each treatment were sampled. The photosynthetic pigment content was measured using ethanol extraction UV spectrophotometer (UV-2600, Shimadzu, Japan) (Bao 2005). Rubisco activity, soluble sugar, starch, soluble protein, and free amino acids were

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measured using enzyme-linked immunosorbent assay kits produced by Shanghai Keshun Biological Technology Co., Ltd. (Shanghai, China). Under the catalysis of Rubisco, one molecule of RuBP combines with one molecule of CO_2 to produce two molecules of 3-phosphoglycerate (PGA). PGA can produce glyceraldehyde-3-phosphate through the action of added 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, and oxidise reduced coenzyme I (NADH), changing the absorbance at 340 nm. The NADH oxidation rate can be calculated, which can ultimately reflect the Rubisco activity (Zha et al. 2021). To measure soluble sugar and starch content, the anthrone colorimetric method was applied; the absorbency at 620 nm wavelength was measured (Luo, Huang 2011). Soluble protein content was determined by bicinchoninic acid (BCA) assay. Under alkaline conditions, cysteine, cystine, tryptophan, tyrosine and peptide bonds in proteins reduce Cu^{2+} to Cu^+, the 2-molecule BCA combines with Cu^+ to form a purple complex with absorption peaks at 540–595 nm, and the highest absorption peak at 562 nm (Wiechelman et al. 1988). The α-amino group of the amino acid reacts with hydrated ninhydrin to produce a blue-violet compound with a characteristic absorption peak at 570 nm. The absorbance at 570 nm was measured to calculate the amino acid content (Wang et al. 2022).

Determination of dry weight. Ten seedlings from each treatment were selected. The aboveground parts were separated from the roots by cutting the stem at ground level. The roots were gently cleaned with a soft brush to remove the soil. To obtain dry weight (DW) measurements, roots and aboveground parts were dried at 105 °C for 30 min and then dried at 70 °C until a constant weight was achieved (Liu et al. 2015).

Determination of nitrogen (N) content. Upper-middle functional leaves were collected from five seedlings in each treatment. The leaves were dried at 105 °C for 30 min and then dried at 70 °C until a constant weight was obtained. A micro-Kjeldahl method was used to digest the leaves with H_2SO_4 to determine the N content (Bao 2005).

Statistical analysis. Treatment means were evaluated using Tukey's multiple-range test ($P < 0.05$). The Spearman correlation method was used to analyse the correlations. All data analyses were performed using SPSS version 23.0 statistical software. Figures and tables were prepared using Microsoft Office 2016.

RESULTS

Net photosynthetic rate (P_n)

By day 27, the P_n of seedlings subjected to the $e2CO_2$ treatment was significantly higher (20.09%) than that of aCO_2-treated seedlings (Figure 1). However, by day 51, the P_n of $e1CO_2$-treated seedlings was significantly higher than that of aCO_2- and $e2CO_2$-treated seedlings and the P_n of $e2CO_2$-treated seedlings was significantly lower than that of aCO_2- and $e1CO_2$-treated seedlings.

Photosynthetic pigment content

By day 27 of treatment with eCO_2, the chlorophyll *b* content of seedlings treated with $e1CO_2$ was significantly higher (8.18%) than that of aCO_2-treated seedlings (Table 1). The chlorophyll *a*, *b*, and *a* + *b* content of $e2CO_2$-treated seedlings was significantly higher than that of aCO_2-treated seedlings, whereas the chlorophyll *a*/*b* content of $e1CO_2$-treated and $e2CO_2$-treated seedlings was significantly lower than that of aCO_2-treated seedlings. By day 51 of treatment with eCO_2, the chlorophyll *a*, *b*, and *a* + *b* content of $e1CO_2$-treated seedlings was significantly higher than that of aCO_2-treated seedlings,

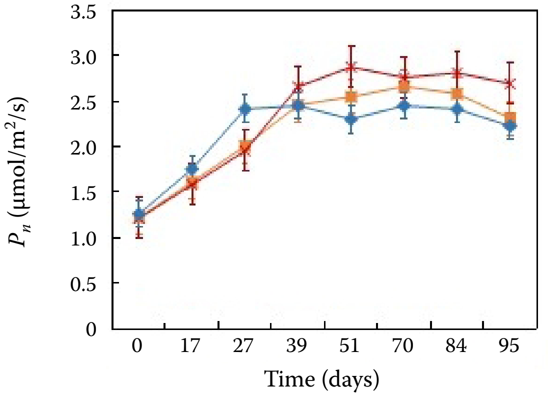

Figure 1. Dynamic response of the net photosynthetic rate (P_n) of *Panax japonicus* seedlings grown under ambient (aCO_2) or elevated ($e1CO_2$ and $e2CO_2$) CO_2 concentrations

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Table 1. Photosynthetic pigment content of *Panax japonicus* grown under ambient (aCO$_2$) or elevated (e1CO$_2$ and e2CO$_2$) CO$_2$ concentrations

Treatments		Chl *a* (mg/g)	Chl *b* (mg/g)	Chl *a* + *b* (mg/g)	Chl *a*/*b*	Car (mg/g)
27 days	aCO$_2$	1.08 ± 0.03^{b}	0.11 ± 0.04^{b}	1.19 ± 0.07^{b}	10.5 ± 1.87^{a}	0.45 ± 0.06^{a}
	e1CO$_2$	1.00 ± 0.02^{b}	0.20 ± 0.06^{a}	1.20 ± 0.05^{b}	4.93 ± 1.34^{b}	0.45 ± 0.07^{a}
	e2CO$_2$	1.21 ± 0.03^{a}	0.18 ± 0.05^{a}	1.41 ± 0.04^{a}	6.62 ± 2.05^{b}	0.47 ± 0.04^{a}
51 days	aCO$_2$	1.13 ± 0.05^{b}	0.18 ± 0.07^{b}	1.32 ± 0.09^{b}	6.83 ± 2.15^{a}	0.47 ± 0.02^{a}
	e1CO$_2$	1.63 ± 0.09^{a}	0.28 ± 0.10^{a}	1.91 ± 0.11^{a}	3.51 ± 0.79^{b}	0.46 ± 0.03^{a}
	e2CO$_2$	0.95 ± 0.04^{c}	0.13 ± 0.03^{b}	1.09 ± 0.04^{b}	7.41 ± 1.43^{a}	0.42 ± 0.03^{a}

Chl – chlorophyll; Car – carotenoid

^{a,b,c}data values relating to the same treatment day with different lowercase letters are significantly different ($P < 0.05$)

whereas the chlorophyll *a* content of e2CO$_2$-treated seedlings was significantly lower (15.93%) than that of aCO$_2$-treated seedlings.

Rubisco activity

By day 27, the Rubisco activity in leaves of e2CO$_2$-treated seedlings was significantly higher (24.10%) than that of aCO$_2$-treated plants; however, the Rubisco activity in leaves of e1CO$_2$- and aCO$_2$-treated seedlings did not differ significantly (Figure 2). By day 51, the Rubisco activity in leaves of e1CO$_2$-treated seedlings was significantly higher (14.83%) than that of aCO$_2$-treated plants, whereas the Rubisco activity of e2CO$_2$-treated plants was significantly lower (14.22%) than that of aCO$_2$-treated plants.

Soluble sugar, starch, soluble protein, and free amino acid content levels

By day 27 of the experiment, the soluble sugar (19.85%) and free amino acid content levels of e1CO$_2$-treated seedlings were significantly higher than that of aCO$_2$-treated seedlings, and the soluble protein, soluble sugar, starch, and free amino acid content levels of e2CO$_2$-treated seedlings were significantly higher than that of aCO$_2$-treated *P. japonicus* (Figure 3). By day 51, the soluble protein, starch, and free amino acid content levels of e1CO$_2$-treated seedlings were significantly higher than that of aCO$_2$-treated seedlings.

Growth parameters

The growth parameters of *P. japonicus* increased over time, and the different treatments showed a time effect on *P. japonicus* growth (Table 2). By day 27, the shoot DW of e2CO$_2$-treated seedlings was significantly greater than that of aCO$_2$- and e1CO$_2$-treated seedlings. By day 51, the stem diameter at ground level, shoot DW, and root DW values of e2CO$_2$-treated seedlings were significantly lower than that of aCO$_2$-treated seedlings.

Nitrogen (N) content

On day 27, the N content of *P. japonicus* leaves of aCO$_2$-, e1CO$_2$-, and e2CO$_2$-treated seedlings did not differ significantly (Figure 4). However, by day 51, the N content of e1CO$_2$-treated seedlings was significantly higher (8.94%) than that of aCO$_2$-treated seedlings, whereas the N content

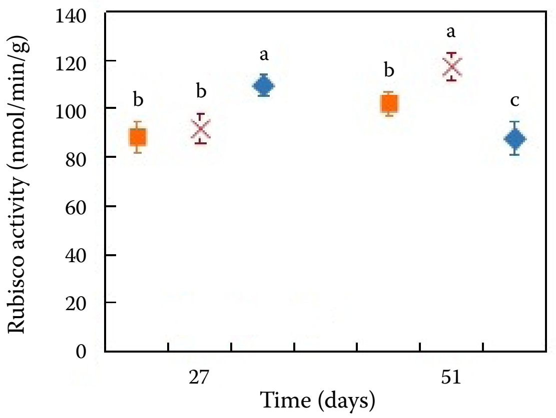

Figure 2. Rubisco activity in leaves of *Panax japonicus* grown under ambient (aCO$_2$) or elevated (e1CO$_2$ and e2CO$_2$) CO$_2$ concentrations

^{a,b,c}data points for the same treatment days with different lowercase letters are significantly different ($P < 0.05$)

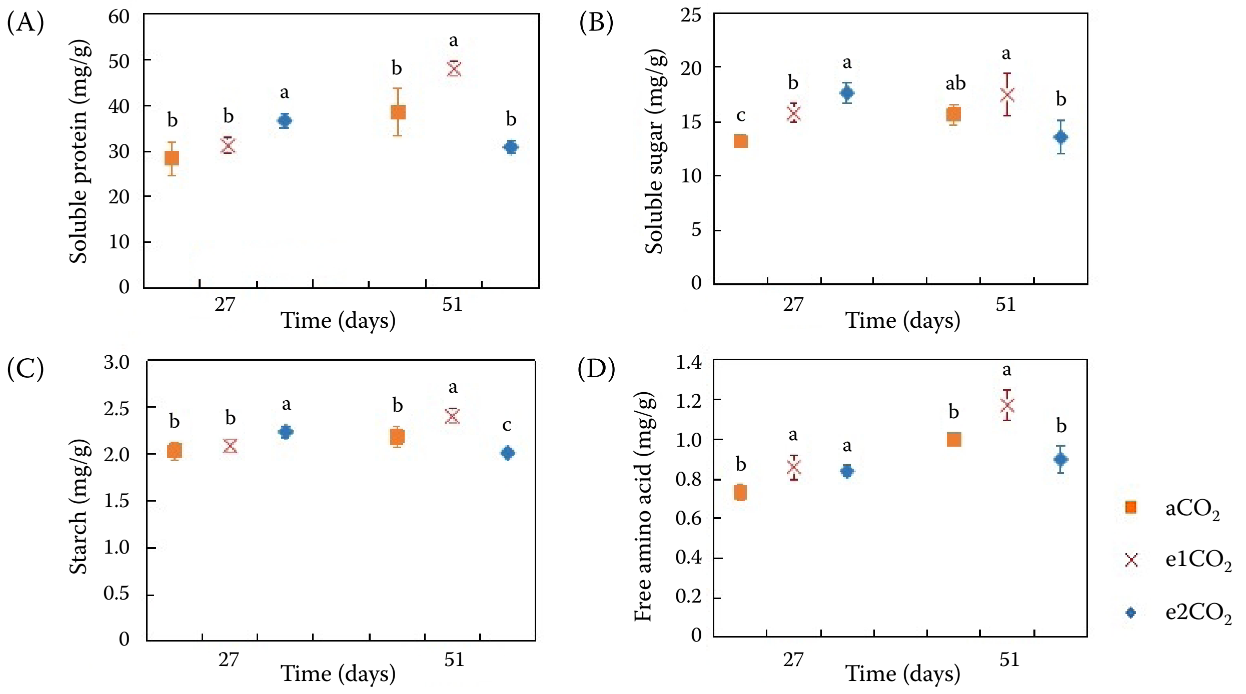

Figure 3. Accumulation of (A) soluble protein, (B) soluble sugar, (C) starch, and (D) free amino acids in the leaves of *Panax japonicus* grown under ambient (aCO_2) or elevated ($e1CO_2$ and $e2CO_2$) CO_2 concentrations

[a,b,c]data points relating to the same treatment day with different lowercase letters are significantly different ($P < 0.05$)

Table 2. Growth parameters of *Panax japonicus* grown under ambient (aCO_2) or elevated ($e1CO_2$ and $e2CO_2$) CO_2 (eCO_2) concentrations

Treatments		Height (cm)	Ground diameter (cm)	Shoot DW (g/plant)	Root DW (g/plant)
27 days	aCO_2	3.06 ± 0.76^a	0.12 ± 0.03^a	0.06 ± 0.01^b	0.61 ± 0.04^a
	$e1CO_2$	2.98 ± 0.44^a	0.11 ± 0.02^a	0.06 ± 0.01^b	0.59 ± 0.03^a
	$e2CO_2$	3.11 ± 0.50^a	0.14 ± 0.02^a	0.09 ± 0.01^a	0.66 ± 0.04^a
51 days	aCO_2	4.90 ± 0.90^{ab}	0.21 ± 0.05^a	0.20 ± 0.01^a	1.05 ± 0.06^a
	$e1CO_2$	5.07 ± 0.99^a	0.22 ± 0.04^a	0.17 ± 0.00^b	0.93 ± 0.03^a
	$e2CO_2$	3.58 ± 0.88^b	0.17 ± 0.02^b	0.14 ± 0.02^c	0.71 ± 0.05^b

DW – dry weight

[a,b,c]data values relating to the same treatment day with different lowercase letters are significantly different ($P < 0.05$)

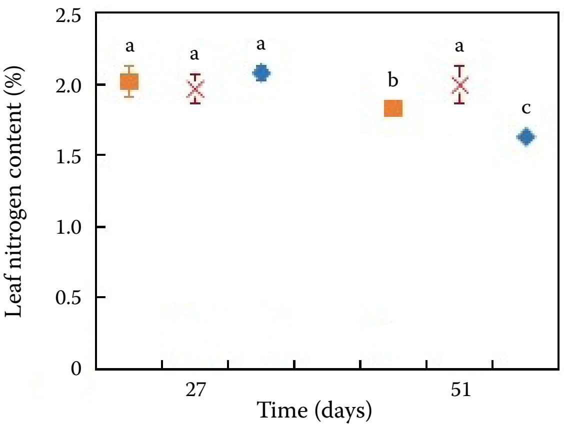

Figure 4. Leaf nitrogen content of *Panax japonicus* seedlings grown under ambient (aCO_2) or elevated ($e1CO_2$ and $e2CO_2$) CO_2 concentrations

[a,b,c]data points relating to the same treatment day with different lowercase letters are significantly different ($P < 0.05$)

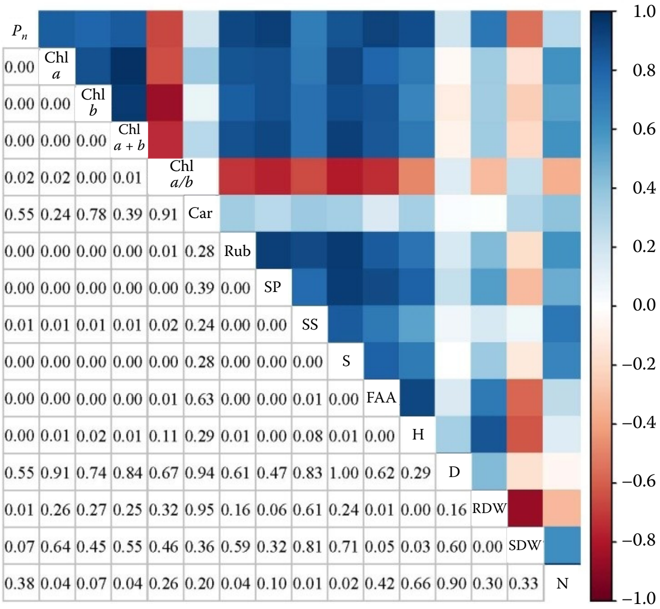

Figure 5. Correlation analysis of different parameters of *Panax japonicus* seedlings
The lower left values are *P* values and the upper right box colours indicate *r* values
P_n – net photosynthetic rate; Chl – chlorophyll; Car – carotenoid; Rub – rubisco activity; SP – soluble protein; SS – soluble sugar; S – starch; FAA – free amino acid; H – height; D – stem diameter at ground level; RDW – root dry weight; SDW – shoot dry weight; N – nitrogen

of e2CO$_2$-treated seedlings was significantly lower (10.91%) than that of aCO$_2$-treated seedlings.

Correlation analysis

Correlation analysis (Figure 5) revealed that the P_n was significantly and positively correlated with Rubisco activity, and with chlorophyll, soluble protein, soluble sugar, starch, and free amino acid content levels, but was not significantly correlated with N content. N content was significantly and positively correlated with Rubisco activity, soluble sugar content, and starch content.

DISCUSSION

Plants grown under conditions of eCO$_2$ can experience significant changes in their histochemistry (Loladze 2002), with a common effect being a reduction in N concentration (Taub, Wang 2008). Interactions involving carbon (C) and N likely modulate terrestrial ecosystem responses to eCO$_2$ levels (Reich et al. 2006). Photosynthesis is the vital metabolism of the plant affected by abiotic stress such as elevated CO$_2$ levels, which ultimately affect the source-sink relationship (Lal et al. 2022). In the short-term, the effect of eCO$_2$ on plant growth is direct and mediated by photosynthesis. In the long-term, the effect of eCO$_2$ is increasingly indirect, with effects occurring through the interaction of, for example, plants, resources (nutrients, water), and temperature (Tuba, Lichtenthaler 2007). Our analyses showed that initially when *P. japonicus* seedlings had been exposed to a CO$_2$ concentration of 550 μmol/mol for 39 days or to 750 μmol/mol for 27 days, the P_n of these seedlings was significantly higher than that of aCO$_2$-treated seedlings. The direct cause of this increase in P_n was an increase in the content of photosynthetic reaction substrates. However, after prolonged exposure to eCO$_2$ conditions, the e1CO$_2$ and e2CO$_2$ treatments had different effects on the P_n of *P. japonicus*. By day 51, the P_n of e1CO$_2$-treated seedlings was still being promoted whereas the P_n of e2CO$_2$-treated seedlings was lower than that of aCO$_2$-treated seedlings. This indicates that growing *P. japonicus* seedlings under moderately eCO$_2$ conditions had a beneficial effect on photosynthesis, whereas a substantial increase in CO$_2$ concentration had a short-term promotional effect and a long-term inhibitory effect on photosynthesis.

Theoretically, the growth of plants under short-term eCO$_2$ conditions may result in an increase in the rate of carboxylation while reducing O$_2$ competition for CO$_2$ at the Rubisco active site, decreasing photorespiration and, thus, increasing photosynthetic efficiency in C3 plants (Galmés et al. 2019). Long-term eCO$_2$ can reduce Rubisco activity through secondary reactions related to carbohydrate over accumulation or reduced C content (Yamori et al. 2012). Our analyses also showed that when *P. japonicus* was subjected to substantially eCO$_2$ conditions, Rubisco activity increased for a short period of time but that ultimately Rubisco activity decreased over time. The photosynthetic pigment, soluble sugar, starch, soluble protein, and free amino acid content levels and growth indices recorded for seedlings in each of the different treatment groups were basically consistent with the trends observed for the P_n, which indicates that a moderately eCO_2 level was beneficial to photosynthesis and the growth of *P. japonicus*, whereas a substantially eCO_2 level had both short- and long-term effects on photosynthesis and growth. A temporal effect of eCO_2 on plant photosynthesis has been reported in numerous studies (Thruppoyil, Ksiksi 2020).

Elevated CO_2 concentrations can reduce N availability in the soil (Tausz-Posch et al. 2019) and can affect N uptake metabolism by plants (Bahrami et al. 2017). Our analyses showed that short-term exposure to eCO_2 had no significant effect on the N concentration of *P. japonicus*, whereas under long-term eCO_2 conditions, the N concentration of *P. japonicus* was significantly increased by moderately eCO_2 conditions, but was significantly decreased by substantially eCO_2 conditions, and photosynthetic efficiency was reduced. The N concentration of *P. japonicus* may have increased under moderately eCO_2 conditions because photosynthesis was promoted, which improved the N uptake metabolism. The photosynthetic efficiency and N content of *P. japonicus* may have decreased under long-term, substantially eCO_2 conditions because eCO_2 affected the metabolism and utilisation of N via photosynthetic physiological processes, such as the synthesis of photosynthetic pigments (Wang et al. 2003), and because eCO_2 affected the photosynthetic efficiency by influencing the availability of N in the plant, and because the influences of these two effects were reciprocal. Given that there was a time effect of eCO_2 on the growth of *P. japonicus*, there was no significant correlation between the N content and the photosynthetic efficiency of *P. japonicus* under eCO_2. However, both the photosynthetic efficiency and the N content were reduced under long-term, substantially eCO_2 conditions. This indicates that N affected the growth of *P. japonicus* in response to eCO_2 conditions mainly when seedlings were subjected to prolonged exposure to eCO_2 conditions.

The effect of long-term eCO_2 on plant growth depends on the balance of supply and demand as well as plant biomass allocation and the source–sink balance during the plant growth period (Jifon, Wolfe 2002). In this study, the adverse effects of substantially eCO_2 conditions on the growth and N nutrition of *P. japonicus* were observed on day 51 of the treatment, which may be attributed to the small size of *P. japonicus* seedlings, and the change in the source–sink balance of *P. japonicus* over time. Under long-term eCO_2 conditions, the organic matter produced by photosynthesis could not be fully utilised by plant growth, resulting in a source–sink imbalance to inhibit growth. Feedback inhibition of carbohydrate accumulation under eCO_2 conditions could also explain why plant growth was affected (Bunce, Sicher 2003).

CONCLUSION

Moderately eCO_2 was beneficial for *P. japonicus* photosynthesis and growth, whereas substantially eCO_2 promoted photosynthesis and growth in the short-term but inhibited photosynthesis and growth in the long-term. Owing to the temporal effect of eCO_2 on the growth of *P. japonicus*, there was no significant correlation between the N content and photosynthetic efficiency of *P. japonicus* under eCO_2; however, the photosynthetic efficiency and N content of *P. japonicus* were reduced under prolonged exposure to substantially eCO_2 conditions. We consider that the long-term inhibitory effect of eCO_2 on the growth of *P. japonicus* can be regulated by N, taking into account the source–sink balance during the growth of *P. japonicus*.

REFERENCES

Adavi S.B., Sathee L. (2021): Elevated CO_2 differentially regulates root nitrate transporter kinetics in a genotype and nitrate dose-dependent manner. Plant Science, 305: 110807.

Bahrami H., De Kok L.J., Armstrong R., Fitzgerald G.J., Bourgault M., Henty S., Tausz M., Tausz-Posch S. (2017): The proportion of nitrate in leaf nitrogen, but not changes in root growth, are associated with decreased grain protein in wheat under elevated CO_2. Journal of Plant Physiology, 216: 44–51.

Bao S.D. (2005): Soil and Agrochemistry Analysis. Beijing, China Agricultural Press. (in Chinese)

Bunce J.A., Sicher R.C. (2003): Daily irradiance and feedback inhibition of photosynthesis at elevated carbon dioxide concentration in *Brassica oleracea*. Photosynthetica, 41: 481–488.

Chang J., Mantri N., Sun B., Jiang L., Chen P., Jiang B., Jiang Z., Zhang J., Shen J., Lu H., Liang Z. (2016): Effects of elevated CO_2 and temperature on *Gynostemma pentaphyllum* physiology and bioactive compounds. Journal of Plant Physiology, 196: 41–52.

Deng Q., Hui D., Luo Y., Elser J., Wang Y.P., Loladze I., Zhang Q., Dennis S. (2015): Down-regulation of tissue N : P ratios in terrestrial plants by elevated CO_2. Ecology, 96: 3354–3362.

Franks P.J., Adams M.A., Amthor J.S., Barbour M.M., Berry J.A., Ellsworth D.S., Farquhar G.D., Ghannoum O., Lloyd J., McDowell N., Norby R.J., Tissue D.T., Von Caemmerer S. (2013): Sensitivity of plants to changing atmospheric CO_2 concentration: From the geological past to the next century. New Phytologist, 197: 1077–1094.

Galmés J., Capó-Bauçà S., Niinemets Ü., Iñiguez C. (2019): Potential improvement of photosynthetic CO_2 assimilation in crops by exploiting the natural variation in the temperature response of rubisco catalytic traits. Current Opinion Plant Biology, 49: 60–67.

Hao X., Li P., Feng Y., Han X., Gao J., Lin E., Han Y. (2013): Effects of fully open-air CO_2 elevation on leaf photosynthesis and ultrastructure of *Isatis indigotica* fort. PLoS ONE, 8: e74600.

IPCC (2014): Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Jifon J.L., Wolfe D.W. (2002): Photosynthetic acclimation to elevated CO_2 in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. Global Change Biology, 8: 1018–1027.

Jin J., Armstrong R., Tang C. (2019): Impact of elevated CO_2 on grain nutrient concentration varies with crops and soils – A long-term face study. Science of the Total Environment, 651: 2641–2647.

Kimball B.A. (2016): Crop responses to elevated CO_2 and interactions with H_2O, N, and temperature. Current Opinion Plant Biology, 31: 36–43.

Lal M.K., Sharma N., Adavi S.B., Sharma E., Altaf M.A., Tiwari R.K., Kumar R., Kumar A., Dey A., Paul V., Singh B., Singh M.P. (2022): From source to sink: Mechanistic insight of photoassimilates synthesis and partitioning under high temperature and elevated CO_2. Plant Molecular Biology, 110: 305–324.

Liu D., Wang J., Li L., Tan H., Ma J., Lu S. (2015): Responses of different peanut cultivars to drought and waterlogging stress and physiological mechanism. Acta Ecologica Sinica, 35: 3817–3824.

Loladze I. (2002): Rising atmospheric CO_2 and human nutrition: Toward globally imbalanced plant stoichiometry? Trends in Ecology & Evolution, 17: 457–461.

Luo W.X., Mi Q., Li W.C., Xu P., Lu Y., Zheng G.W., Chen J. (2023): The response of *Polygonatum kingianum* to long-term CO_2 elevation. Molecular Plant Breeding, 12: 1–12.

Luo X., Huang Q. (2011): Relationships between leaf and stem soluble sugar content and tuberous root starch accumulation in cassava. Journal of Agricultural Science, 3: 64–72.

Ng C.W.W., Wang Y.C., Ni J.J., Tsim K.W.K. (2022): Coupled effects of CO_2 and biochar amendment on the yield and quality of *Pseudostellaria heterophylla*. Industrial Crops and Products, 188: 115599.

Norby R.J. (2021): Comment on “Increased growing-season productivity drives earlier autumn leaf senescence in temperate trees.” Science, 371: eabg1438.

Qiang Q., Gao Y., Yu B., Wang M., Ni W., Li S., Zhang T., Li W., Lin L. (2020): Elevated CO_2 enhances growth and differentially affects saponin content in *Paris polyphylla* var. *yunnanensis*. Industrial Crops and Products, 147: 112124.

Reich P.B., Hungate B.A., Luo Y. (2006): Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annual Review of Ecology Evolution and Systematics, 37: 611–636.

Ren J., Guo S., Xu C., Yang C., Ai W., Tang Y., Qin L. (2014): Effects of different carbon dioxide and led lighting levels on the anti-oxidative capabilities of *Gynura bicolor DC*. Advances in Space Research, 53: 353–361.

Ripple W.J., Wolf C., Gregg J.W., Levin K., Rockström J., Newsome T.M., Betts M.G., Huq S., Law B.E., Kemp L., Kalmus P., Lenton T.M. (2022): World scientists’ warning of a climate emergency. BioScience, 72: 1149–1155.

Sharma S., Walia S., Rathore S., Kumar P., Kumar R. (2020): Combined effect of elevated CO_2 and temperature on growth, biomass and secondary metabolite of *Hypericum perforatum* L. in a western Himalayan region. Journal of Applied Research on Medicinal and Aromatic Plants, 16: 100239.

Taub D.R., Wang X. (2008): Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. Journal of Integrative Plant Biology, 50: 1365–1374.

Tausz-Posch S., Tausz M., Bourgault M. (2019): Elevated CO_2 effects on crops: Advances in understanding acclimation, nitrogen dynamics and interactions with drought and other organisms. Plant Biology, 22: 38–51.

Thruppoyil S.B., Ksiksi T. (2020): Time-dependent stomatal conductance and growth responses of *Tabernaemontana divaricata* to short-term elevated CO_2 and water stress at higher than optimal growing temperature. Current Plant Biology, 22: 100127.

Tuba Z., Lichtenthaler H.K. (2007): Long-term acclimation of plants to elevated CO_2 and its interaction with stresses. Annals of NewYork Academy of Sciences, 1113: 135–146.

Wang K.Y., Kellomäki S., Zha T. (2003): Modifications in photosynthetic pigments and chlorophyll fluorescence in 20-year-old pine trees after a four-year exposure to carbon dioxide and temperature elevation. Photosynthetica, 41: 167–175.

Wang X., Wei X., Wu G., Chen S. (2021): Ammonium application mitigates the effects of elevated carbon dioxide on the carbon/nitrogen balance of *Phoebe bournei* seedlings. Tree Physiology, 41: 1658–1668.

Wang Z., Ma S., Li L., Huang J. (2022): Effect of wheat bran dietary fiber on structural properties and hydrolysis behavior of gluten after synergistic fermentation of *Lactobacillus plantarum* and *Saccharomyces cerevisiae.* Frontiers in Nutrition, 9: 982878.

Wiechelman K.J., Braun R.D., Fitzpatrick J.D. (1988): Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. Analytical Biochemistry, 175: 231–237.

Yamori W., Masumoto C., Fukayama H., Makino A. (2012): Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. The Plant Journal, 71: 871–880.

Zhang S., Zhang L. (2022): Using an IR camera to improve leaf area and temperature measurements: A new method for increasing the accuracy of photosynthesis-related parameters. Agricultural and Forest Meteorology, 322: 109005.

Zha Q., Xi X., He Y., Yin X., Jiang A. (2021): Effect of short-time high-temperature treatment on the photosynthetic performance of different heat-tolerant grapevine cultivars. Photochemistry and Photobiology, 97: 763–769.

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