Effects of urea-N and CO_2 coupling fertilization on the growth, photosynthesis, yield and anthocyanin content of hydroponic purple cabbage *Brassica campestris ssp.* chinensis

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Abstract: Water and fertilizer coupling is a high-efficiency technology for the development of facility agriculture. However, the interaction effect of nitrogen (N) and air carbon dioxide (CO_2) on hydroponic purple cabbage, especially on its leaf anthocyanins under hydroponic solution systems, remains unexplored. In this study, six treatments were set as C0N0, C0N2, C0N4, C1N0, C1N2 and C1N4, with N0, N2 and N4 being 0.0 g/L, 0.2 g/L and 0.4 g/L exogenous urea-N to hydroponic solution dilution, respectively. C0 and C1 were set as with and without CO_2 fertilizer (i.e., 800 g CO_2 agent added one week after transplanting and 600 g CO_2 agent added when the plant reached 15 cm in height), respectively. Pot experiments were conducted to investigate the interaction effect of N and air CO_2 (N×CO_2) on the growth, photosynthesis, yield and anthocyanin content of hydroponic purple cabbage *Brassica campestris ssp.* chinensis. The results showed N×CO_2 extremely significantly influenced plant height (*H*), net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), intercellular oxidation concentration (*Ci*), transpiration rate (*Tr*), leaf water use efficiency (*LWUE*) and yield. The C1N0 treatment had the largest yield at 262.5 g/plant, with higher values for root length, root weight, plant height and leaf number than the other treatments. The *Pn*, *Ci* and *Tr* of C1N4 were the highest at 3.05 μmol $CO_2/m^2{\cdot}s$, 352.8 μmol $CO_2/m^2{\cdot}s$ and 2.31 mmol $H_2O/m^2{\cdot}s$, respectively. The C1N2 treatment received the largest *Gs* value of 0.70 mol $H_2O/m^2{\cdot}s$ and the largest *Tr* of 2.31 mmol $H_2O/m^2{\cdot}s$. There was the highest LWUE for C0N2 (1.41) and the highest anthocyanin content for C1N2 (1.35 mg/kg). There was a significant negative correlation between leaf number and anthocyanin (r = –0.414, p<0.05). The findings demonstrated that adding CO_2 fertilizer and 0.2 g/L exogenous urea-N to hydroponic solution dilution is a potential N×CO_2 coupling strategy to increase anthocyanin and the yield of purple cabbage.

Keywords: nitrogen concentration, carbon dioxide, purple cabbage, hydroponic solution, anthocyanins
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1 Introduction

Facility agriculture has been playing an important role in China's economy, taking a significant part in providing sufficient food for the growing population[1]. Greenhouse vegetable production has expanded rapidly in recent years, but the sustainability of planting methods has been a major problem[2]. To ensure the sustainable development of protected agriculture in China, effective environmental management was a measure to achieve high quality and high yield of vegetables in the greenhouse[3]. Hydroponic culture (HC) has been around for decades as one of the planting methods with effective environmental management to grow plants, the success key of which is the configuration of hydroponic nutrient solution (HNS) and oxygen dissolved in the water at levels that lead to high rates of plant growth[4]. Parameter adjustments in HNS can affect crop growth and yield to some extent[5].

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Nitrogen (N) is an important nutrient element necessary for plant growth and development[6]. A large amount of N, as a very important element in HNS, was often applied to supply N absorption for the growth and quality of leafy vegetables[7-9]. Increasingly pronounced differences in dry matter accumulation in hydroponic lettuce have been reported over time under different N treatments, with higher N concentrations leading to higher N uptake[8]. Mahlangu et al.[10] found 120 mg/L N in nutrient solution could get the largest fresh weight of lettuce crown. Moreover, Chinese cabbage and lettuce were considered to grow best in nutrient solutions with an N concentration of 112 mg/L[11]. However, excessive N application will cause many negative effects[12,13]. Excessive N will increase the nitrate content in vegetables, among which nitrate and nitrite are extremely harmful to human health[14]. Thus, different forms and dosages of N fertilizer added in HNS had different important impacts on the growth of leafy vegetables[15]. Single nitrate nitrogen (NO_3^--N) in nutrient solution reduced the chlorophyll synthesis and photosynthesis of lettuce seedlings, while single ammonium nitrogen (NH_4^+-N) in nutrient solution affected the growth of lettuce seedlings, resulting in lettuce buds and roots growth[16]. Meanwhile, NH_4^+-N could dissipate excess light energy, significantly reducing the self-protection ability, the actual photochemical efficiency, strong seedling index, and leaf transpiration[17]. However, it increased leaf water use efficiency, maintain high carboxylation efficiency, and then improved the net photosynthetic rate (*Pn*) of pepper seedling leaves[17]. Besides, reasonable N in HNS could reduce environmental pollution caused by N losses[18].

Water and N management is not only essential for crop yield but also contributes to greenhouse gas (GHG) emissions[19,20]. The rising air carbon dioxide (CO_2, a greenhouse gas) is a particularly important issue in agriculture, horticulture and local plant production. Since CO_2 had complex effects on plant growth and photosynthetic activity with many factors, facility greenhouses (especially insulated greenhouses) seriously lacked air CO_2, even close to or reaching the CO_2 compensation point[21]. CO_2 enrichment was a common technique in vegetable cultivation. Compared with plants grown under normal environmental CO_2, plants grown under higher CO_2 concentrations had lower leaf spread and biomass[22]. Long-term elevated CO_2 could more greatly decrease leaf dry weight, photosynthesis rate, stomatal conductance and internal CO_2 concentration of Chinese cabbages[23].

The increased plant growth depended on the nature of common limiting factors, especially the availability of nutrients[18]. Multi-factors coupling effect is an important aspect of agricultural production. Water-fertilizer coupling technology has been widely used in the world, followed by the water-dissolved oxygen and water-temperature coupling model in greenhouses[24]. It is also necessary to study the mechanism of the comprehensive regulation of water and air CO_2 in the greenhouse. With a certain amount of N fertilizer applied, CO_2 enrichment increased the number of fine roots and leaves, whereas it reduced the N uptake and impaired plant growth[25]. Furthermore, applying more soil ammonium nitrogen (NH_4^+-N) was more conducive to improving the response of plants to CO_2 fertilization[26]. Additionally, N management and air CO_2 could also affect the quality of Chinese cabbage (e.g., leaf color) [27]. Moreover, anthocyanin (*OPC*) as one of the important pigments to determine the purple color of crops to attract pollinators and seed dispersers[28], had an increasing application potential for its function in human health. The sales volume and price of purple leafy vegetables in the Chinese market increased, and the price of organic purple leafy vegetables could reach 50-60 CNY/kg[28]. The N limitation and air CO_2 may influence anthocyanin accumulation[29,30]. Our former research showed the increased air CO_2 concentration and reduced N content in hydroponic solution significantly decreased *OPC* of purple romaine lettuce[28]. However, the interaction effects of N and air CO_2 (N×CO_2) on purple cabbage (*Brassica campestris ssp.* chinensis)[31], especially on *OPC* of its leaves under a hydroponic solution system, remain unexplored. It was hypothesized that the interaction between appropriate N formation in hydroponic solution and certain air CO_2 concentrations could promote purple cabbage growth and leaf *OPC*.

In this study, three concentration gradients of exogenous urea-N (i.e., 0.0 g/L, 0.2 g/L and 0.4 g/L) and two types of CO_2 application (i.e., with and without CO_2 fertilizer adding) were selected, and their interaction was investigated by pot experiments on the growth, photosynthesis, yield and anthocyanin content of hydroponic purple cabbage *Brassica campestris ssp.* chinensis. The objectives were to: 1) study the N×CO_2 effects on agronomic growth, leaf photosynthesis, yield and *OPC* of hydroponic purple cabbage; 2) optimize suitable N×CO_2 coupling strategy to gain higher yield and *OPC*; 3) investigate the relationships between growth, photosynthesis and *OPC*. This study will provide a theoretical basis for exploring the efficient use of carbon and nitrogen coupling in facility agriculture.

2 Materials and methods

2.1 The tested site

The tested site was located in the College of Horticulture, Jinling Institute of Technology (N 32.1234°, E 118.8111°), Nanjing city, Jiangsu province, China. Two indoor plastic sheds were built in a multi span glass greenhouse, each with 2.0 m ridge height, 1.6 m eaves height, 4 m length and 2 m width. All sides and bottom were covered with plastic films, only one side could be opened when necessary.

2.2 Experimental treatments and management

The hydroponic planting was conducted in a pipeline at 63 mm diameter, with planting holes at 32 mm diameter. The variety of purple cabbage was violet *Brassica campestris* F1 from Jiangsu Academy of Agricultural Science. Six N and CO_2 coupling treatments were set as C0N0, C0N2, C0N4, C1N0, C1N2 and C1N4, with four repetitions. The N0, N2 and N4 were designed as hydroponic solution diluent stock solution (HSDSS) without exogenous N, 10 L HSDSS with 2 g urea-N added, and 10 L HSDSS with 4 g urea-N added, respectively. The hydroponic solution should be renewed every 7-10 d according to plant growth, and it was circulated regularly from 8:00 a.m. to 6:00 p.m. All solutions were maintained at the temperature of 21.5°C, with pH at 7.5 (N0), 7.4 (N2) and 7.2 (N4), respectively. The salinity contents were at 1667 mg/L (N0), 2198 mg/L (N2) and 2992 mg/L (N4), respectively. The HSDSS was made by hydroponic solution stock solution:water=1:3. The hydroponic solution stock solution contained 190.62 g N (NO_3^--N: NH_4^+-N =1:8.6), 70.15 g P_2O_5, 279.22 g K_2O, 80.12 g MgO, 217.35 g CaO, 64.35 g S, 5.20 g Fe, 0.49 g B, 0.67 g Mn, 0.045 g Zn, 0.02 g Cu, 0.007 g Mo per 0.5 L. The self-made quick acting urea-N was from Jiangsu Academy of Agricultural Science with N at 42%. C0 and C1 meant no CO_2 agent and adding CO_2 agent, respectively. C1 was conducted by adding 800 g CO_2 agent a week after transplanting. When plant height reached 15 cm, 600 g CO_2 agents were added again. The CO_2 agent was a powder solid with the brand "Quzhouguosheng" in China[28]. The field seedling was conducted on September 9th, 2020, and then

8 cm plants were transplanted on October 28th.

2.3 Items and methods

2.3.1 Properties of hydroponic solution

The temperature (*T*), pH and salinity of the hydroponic solution before planting and replacement were tested by an operation manual pH meter (ATC, China) and salinometer (YD-1H, China) from reference [28].

2.3.2 Air carbon dioxide (CO_2) monitor

The air CO_2 was continuously monitored at an interval of 10 min by an intelligence CO_2 monitor (Lvkedu, LKD-RMU series, model 597, China) from reference [28]. The air CO_2 during the whole growth period was investigated with max, min and average values of 631 mg/kg, 390 mg/kg and 456 mg/kg under C0. They were 1709 mg/kg, 405 mg/kg and 710 mg/kg under C1.

2.3.3 Growth indexes of plant

The root length (*RL*) and plant height (*H*) were measured at harvest time, by using a ruler with 0.1 accuracies. The number of leaves (*NL*) of each plant was recorded, while root weight (*RW*) and yield (*Y*) were weighed by an electronic balance at 500 g maximum weighing range with 0.1 accuracies.

2.3.4 Photosynthetic indexes

The net photosynthetic rate (*Pn*), stomatal conductance (*gs*), intercellular oxidation concentration (*Ci*), and transpiration rate (*Tr*) of leaves were tested by LI 6400 portable photosynthesis system (LI-COR Corporate, U.S.A,) at harvest time, respectively. Leaf water use efficiency (*LWUE*) was calculated by the ratio of *Pn* and *Tr*[32].

2.3.5 Anthocyanin (*OPC*)

The anthocyanin (*OPC*) of leaves was extracted by using 0.1 mol/L hydrochloric acid ethanol solution[28,33], and then measured by spectrophotometric method. Firstly, 2 g of fresh leaves were put into a triangular flask with 10 mL 0.1 mol/L hydrochloric acid ethanol solution added, and then crushed to a certain extent. After, all triangular flasks were immersed in a 60°C water bath for an hour. Then, they were cooled for about an hour. The cooled liquid from each triangular flask was put into a 25 mL volumetric flask with 5 mL 0.1 mol/L hydrochloric acid ethanol solutions added lasting for 15 min. 0.1 mol/L hydrochloric acid ethanol solution at 5 mL was added to each volumetric flask lasting for 15 min again. Finally, each volumetric flask was dissolved in 25 mL of distilled water. The optical density value (*OD*) of each solution was determined by a spectrophotometer (PHILes, D-8, Nanjing Feile Instrument Co., Ltd., China) at 530 nm, 620 nm, 650 nm wavelength, using 0.1 mol/L hydrochloric acid ethanol solution as the reference solution. The content of *OPC* was calculated by the following Equations (1) and (2)[28].

$$OD = (OD_{530} - OD_{620}) - 0.1(OD_{650} - OD_{620}) \quad (1)$$

$$OPC = OD\varepsilon V \times 1000m \times 0.6547 \quad (2)$$

where, OD_{530}, OD_{620} and OD_{650} mean *OD* at 530 nm, 620 nm, 650 nm wavelength; ε Is molar absorption coefficient of anthocyanin (4.62×106); *v* is the total extract liquid volume, mL; *m* is the leaf quality, g. The unit of OPC is mg/kg.

2.4 Statistical analysis

Statistical differences at 0.05 level among individual treatments were analyzed by using Duncan's multiple comparison tests (one-way ANOVA) in IBM SPSS Statistics v.17.0 (IBM Corp., Armonk, NY, USA), by which variance analysis of N, CO_2 and their interaction (N ×CO_2) effects were analyzed.

3 Results

3.1 Plant growth

Figure 1 shows that N in water planting solution and air CO_2 had significant effects on *RL*, *RW*, *H* and *NL* of purple cabbage (p<0.05), respectively. The treatment C1N0 significantly got the largest *RL* at 32.6 cm (p<0.05, Figure 1a). However, C0N4 and C1N4 obviously received the shortest *RL* at 15.5 cm and 19.3 cm (p<0.05), respectively. C1N0 obtained the largest *RW* at 14.9 g/plant (Figure 1b), not evidently differing from C0N0 (14.0 g/plant), both of which were significantly heavier than the rest (p<0.05). Moreover, C0N4 and C1N4 obviously received the smallest *RW* at 4.9 g/plant and 6.7 g/plant (p<0.05), respectively. Figure 1c shows that C1N2 got the highest plant *H* at 23.1 cm, but not evidently differing from C1N0 and C0N0 (p>0.05). C0N4 obviously had the shortest *H* just at 12.8 cm (p<0.05). C1N0 also had the significant most *NL* at 12.4 piece/plant (p<0.05, Figure 1d). However, C0N2 and C0N4 obtained the smallest leaves at 9.0 piece/plant and 9.2 piece/plant, respectively.

3.2 Leaf photosynthesis

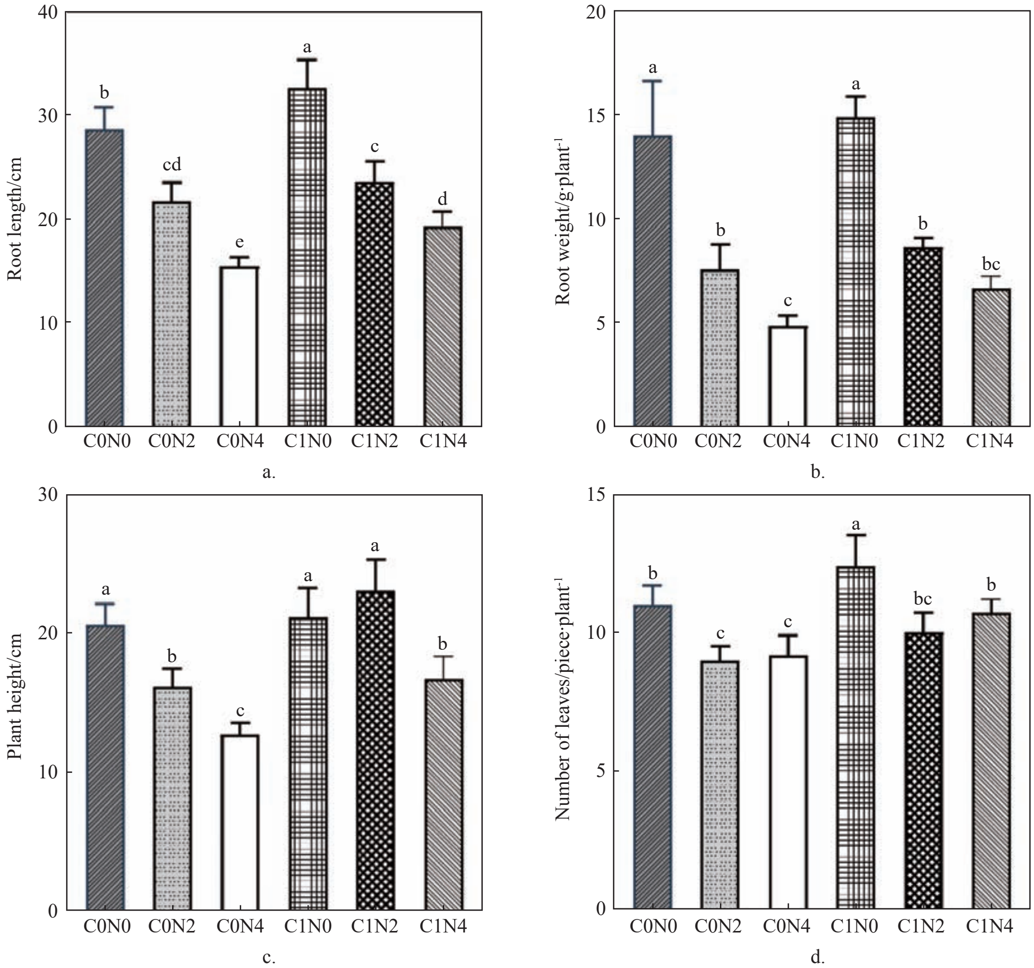
The results of *Pn*, *gs*, *Ci*, *Tr* and *LWUE* are shown in Figure 2, each with a significant difference among all treatments (p<0.05). The treatment C1N4 got the highest *Pn* at 3.05 μmol $CO_2/m^2\cdot s$, obviously differing from others (p<0.05). However, C1N2 received the lowest *Pn* at 2.42 μmol $CO_2/m^2\cdot s$, just not obviously differing from C0N0, C0N2 and C0N4 (p>0.05). C1N2 also received the biggest *gs* value at 0.70 mol $H_2O/m^2\cdot s$, just significantly differing from C1N4, C0N0 and C0N2 (p<0.05). C0N2 met with the minimum *gs* value at 0.47 mol $H_2O/m^2\cdot s$. C1N4 obtained the largest *Ci* value at 352.8 μmol $CO_2/m^2\cdot s$, followed by C1N2 at 351.3 μmol $CO_2/m^2\cdot s$ and C1N0 at 349.5 μmol $CO_2/m^2\cdot s$, respectively. They obviously surpassed the rest treatments (p<0.05). Moreover, C0N0 got the lowest *Ci* at 313.3 μmol $CO_2/m^2\cdot s$. C1N2 also got the biggest *Tr* value at 2.31 mmol $H_2O/m^2\cdot s$, not obviously a discrepancy with C0N4 and C1N4 (p>0.05). C0N2 showed the significant smallest *Tr* at 1.78 mmol $H_2O/m^2\cdot s$. Significantly, C0N0 got a lower *Tr* less than 2.00 mmol $H_2O/m^2\cdot s$ (p<0.05). Considered with *Pn* and *Tr*, C0N2 and C1N4 had the largest *LWUE* values at 1.41 and 1.40, respectively. C1N2 had the smallest *LWUE* at 1.05. Furthermore, there was no obvious discrepancy in *LWUE* between C0N0, C0N2, C1N0 and C1N4 (p<0.05), the same as C1N2 and C0N4.

3.3 Yield

Figure 3 shows there was a significant difference in *Y* values existed between all treatments (p<0.05). No matter under what kind of CO_2 treatment, *Y* decreased as the N concentration in the nutrient solution increased. It was worth noting that each value of *Y* under C1 was higher than that under C0 in the same N solution. The treatment C1N0 received the largest *Y* at 262.5 g/plant, obviously being higher than the rest values (p<0.05). The second largest *Y* at 232.5 g/plant presented in C1N2, followed by C0N0 at 210.0 g/plant. The rest yields were both lower than 200.0 g/plant, with the lowest *Y* obviously under C0N4 at 75.0 g/plant.

3.4 Anthocyanin

Figure 4 shows a significant divergence of leaf *OPC* existed among all treatments (p<0.05). *OPC* in leaves increased as N concentration in nutrient solution went up under C0. However, *OPC* increased at first and then decreased as N concentration increased under C1. C1N2 and C0N4 received the first and second largest values of *OPC* at 1.35 mg/kg and 1.23 mg/kg, respectively. They



Note: The lowercase letters in the same column indicate that the difference between treatments was significant at $p<0.05$ with Duncan's multiple range test.

Figure 1 Conditions of different growth indexes under N×CO_2 treatments (n=4)

were both significantly bigger than C0N0, C1N0 and C1N4 ($p<0.05$). The third biggest *OPC* existed in C0N2 at 1.10 mg/kg, just obviously surpassing C1N0 ($p<0.05$). Therefore, C1N0 significantly received the lowest *OPC* value just at 0.73 mg/kg ($p<0.05$).

3.5 Conditions of correlation analysis between indexes

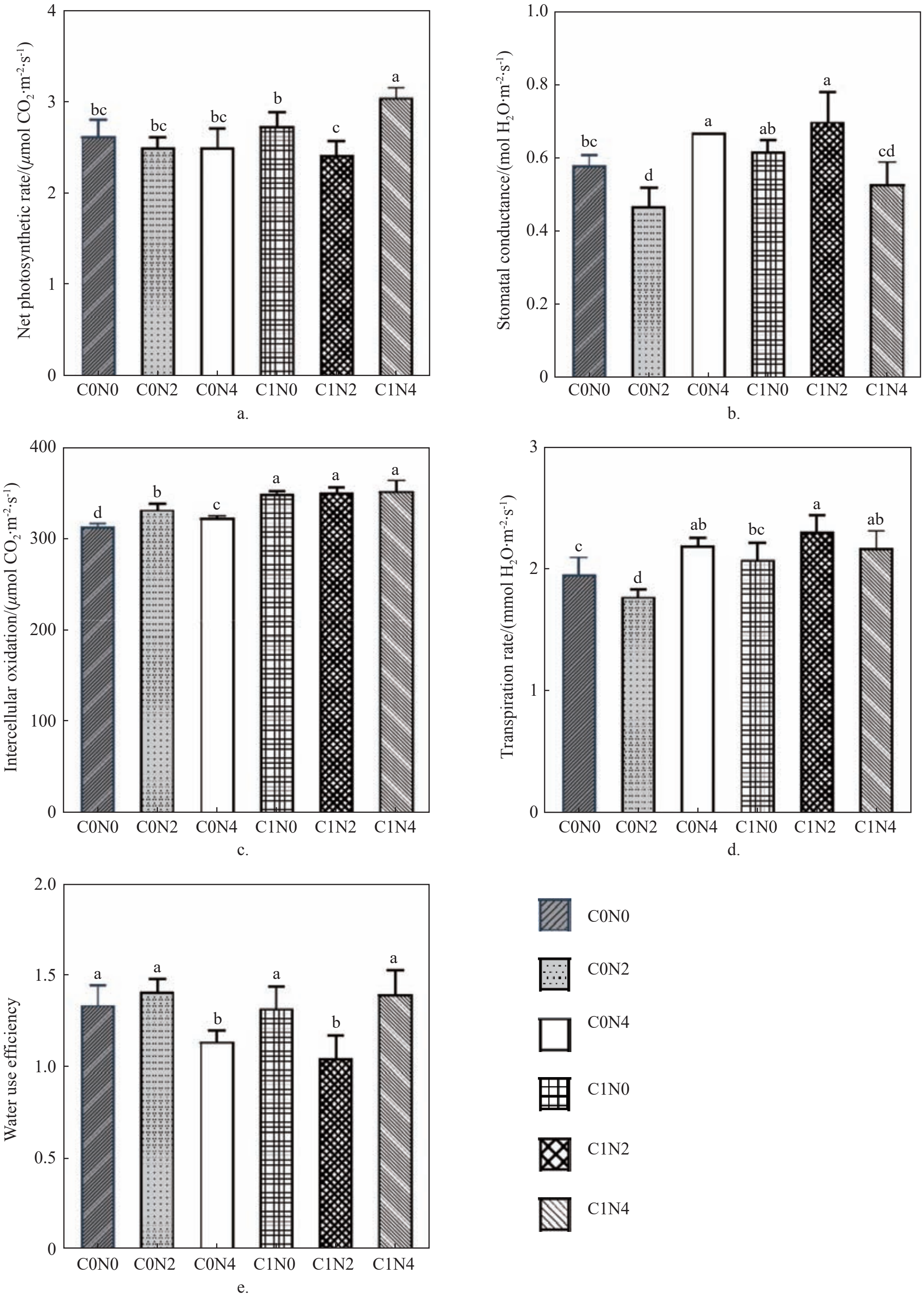
Figure 5 shows that *Pn* had an extremely significant correlation with *LWUE* (r=0.645, $p<0.01$). The *gs* got an extremely significant correlation with *Tr* (r=0.684, $p<0.01$), and an extremely negative significant correlation with *LWUE* (r = –0.739, $p<0.01$). *Tr* had an extremely negative obvious correlation with *LWUE* (r = –0.710, $p<0.01$). *Ci* had no obvious correlation with others ($p>0.05$). *RL* extremely significantly correlated with *RW* (r=0.921, $p<0.01$), *H* (r=0.711, $p<0.01$), *NL* (r=0.675, $p<0.01$) and *Y* (r=0.860, $p<0.01$), respectively. But it also had a negative significant correlation with *OPC* (r = –0.414, $p<0.05$). *RW* extremely significantly correlated with *H* (r=0.627, $p<0.01$), NL (r=0.698, $p<0.01$) and *Y* (r=0.779, $p<0.01$). *H* extremely significantly correlated with NL (r=0.531, $p<0.01$) and *Y* (r=0.895, $p<0.01$). There was an extremely significant relationship between *NL* and *Y* (r=0.701, $p<0.01$).

4 Discussion

4.1 Plant growth and yield

The greenhouse microclimate control is crucial to the proper growth of the crops, impacting their yield, productivity, quantity, quality, and various diseases[34]. The N in HNS and air CO_2 were two effective environmental factors influencing leaf vegetable growth[18]. A certain N concentration was believed to obtain a higher yield and quality of hydroponic crops[9]. N forms had a certain inhibitory effect on plant growth, as NH_4^+-N alone would poison the plant and make it wilt, negatively affecting the rhizosphere[16]. Meanwhile, NO_3^--N alone would cause excessive nitrate accumulation in the plant. Therefore, mixtures of NH_4^+-N and NO_3^--N at a certain ratio in HNS would be superior to single-form nutrient solutions for crop growth[35]. For example, reducing the ratio of NO_3^--N: NH_4^+-N to some extent would significantly decrease plant growth and biomass[36]. Additionally, the increase in CO_2 concentration added to the number of plant leaves[22]. But, elevated CO_2 also could decrease the leaf dry weight of some Chinese cabbages[23]. Remarkably, the interaction between appropriate nutrient concentration and CO_2 (N×CO_2) would increase the number of fine roots and leaves and decrease N uptake to harm plant growth[25].

As one of the Chinese small cabbage varieties, purple cabbage in HC was increasingly popular in the market at a higher price. In this study, it was found that N in HNS and air CO_2 had obvious effects on purple cabbage growth. The treatment C1N0 significantly got the largest values of *RL* at 32.6 cm, *RW* at 14.9 g/plant and *NL* at 12.4 piece/plant ($p<0.05$, Figure 1). The highest *H* at 23.1 cm was presented in C1N2. The treatments C0N4 and C1N4 received the shortest *RL*s at 15.5 cm and 19.3 cm, with the obvious lightest *RW* at 4.9 g/plant and 16.7 g/plant, respectively. The shortest *H* at 12.8 cm was under C0N4, with the smallest *NL*s both under C0N2 and C0N4, respectively. The largest *Y* at 262.5 g/plant presented in

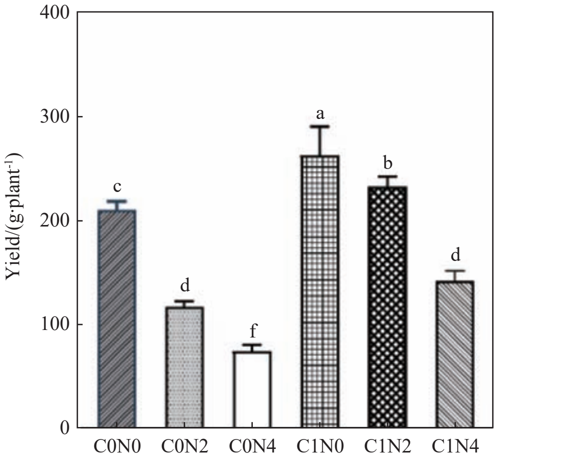


Note: The lowercase letters in the same column indicate that the difference between treatments was significant at $p<0.05$ with Duncan's multiple range test.

Figure 2 Conditions of leaf photosynthesis indexes under N×CO_2 treatments (n=4).

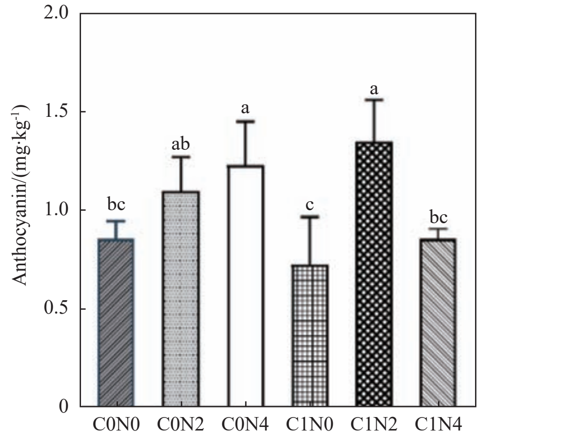
C1N0, with the lowest Y just at 75.0 g/plant in C0N4. The Pearson correlation analysis results (Figure 5) showed *RL*, *RW*, *H* and *NL* had extremely significant positive correlations with *Y* ($p<0.01$), which conformed to the general growth law. The variance analysis results (Table 1) demonstrated that N in HNS had extremely significant effects on *RL*, *RW*, *H*, *NL* and *Y* ($p<0.01$). CO_2 fertilizer had extremely evident effects on *RL*, *H*, *NL* and *Y* ($p<0.01$). However, N×CO_2 just only extremely significantly affected *H* and *Y* ($p<0.01$). These results demonstrated adding CO_2 fertilizer could enhance purple cabbage growth, while extra urea N in HNS did not improve plant growth. It might be because of air CO_2 in the whole growing period and the changed properties of hydroponic solution at harvest time (Table 2).

Low or high temperature (*T*) could affect the rate of nutrient uptake by crop roots[24]. However, a report showed high salinity of about 2000 and 2500 mg/L could significantly increase *RW*, *NL* and *Y* of a medicinal plant[37]. Higher salinity could harm crop growth, but the increased N fertilizer application could alleviate the salt inhibition and promote the growth of processed tomatoes[38]. The pH level of HC affects nutrient availability just as much as the actual amount of nutrients present in the solution[4]. The N form most influenced pH shifting in the solution. Nitrate taken up by plants



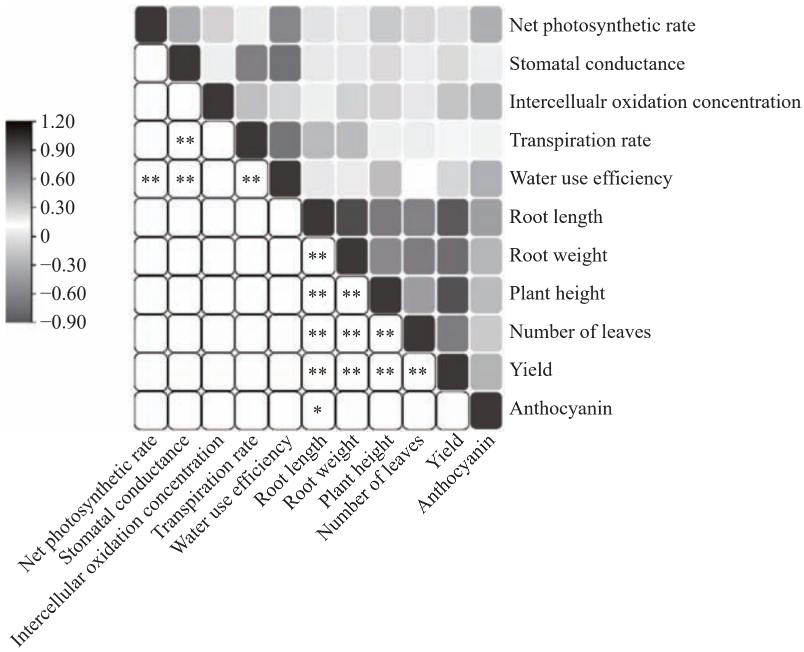
Note: The lowercase letters in the same column indicate that the difference between treatments was significant at $p<0.05$ with Duncan's multiple range test.

Figure 3 Conditions of yield (Y) under N×CO_2 treatments (n=4)



Note: The lowercase letters in the same column indicate that the difference between treatments was significant at $p<0.05$ with Duncan's multiple range test.

Figure 4 Conditions of anthocyanin (OPC) between N×CO_2 treatments (n=4)



Note: **and *displayed Pearson correlation at 0.01 and 0.05 significant levels, respectively.

Figure 5 Conditions of Pearson correlation analysis between all indexes

Table 1 Conditions of different growth indexes under N×CO_2 treatments (n=24)

Factor	RL	RW	H	NL	Yield
N	**	**	**	**	**
CO_2	**	ns	**	**	**
N×CO_2	ns	ns	**	ns	**

Note: ** and * displayed extremely significant differences at 0.01 and 0.05 levels, respectively. The ns displayed no significant difference.

Table 2 Conditions of HC liquid under N×CO_2 treatments (n=3)

Treatment	Salinity/mg·L⁻¹	T/°C	pH
C0N0	1066.5±23.2b	21.9±1.7a	8.1±0.1a
C0N2	1134.5±16.9ab	21.8±1.0a	8.2±0.1a
C0N4	1126.5±26.1ab	21.6±1.1a	8.1±0.1a
C1N0	1116.0±20.0ab	21.4±1.2a	8.1±0.2a
C1N2	1091.0±71.5b	21.1±0.0a	8.1±0.0a
C1N4	1170.0±18.7a	21.4±1.2a	8.2±0.0a

Note: The lowercase letters in the same column indicate that the difference between treatments was significant at $p<0.05$ with Duncan's multiple range test.

could increase the pH, while ammonium use reduced it. Therefore, using both nitrate and ammonium at a ratio could maintain the pH changes[4]. In this paper, the initial values of salinity in our hydroponic solution showed N0<N2<N4, with pH having the reverse order. Remarkably, the harvested T and pH under all treatments almost had no differences among treatments (Table 2), while salinity in hydroponic significantly differed among treatments. At harvest time, the reduced salinities under all treatments were in descending order as C0N4, C1N4, C1N2, C0N2, C0N0 and C1N0. The largest Y agreed with the smaller changes in solution salinity, displaying that a high salt nutrient solution could harm purple cabbage growth. Meanwhile, the changed air CO_2 under C0 and C1 during the whole growing period might be the other reason to improve purple cabbage growth. That was also to say N and CO_2 had the interaction effects on these indexes.

4.2 Leaf photosynthesis

Photosynthesis is a biological process of converting light energy into chemical energy by plants, synthesizing and effectively accumulating organic matter. So, enhancing photosynthesis was an effective means to improve vegetable yield and quality[39]. N was believed as a factor which could directly or indirectly affect photosynthesis[40]. Single NH_4^+-N in HNS had been believed to affect the growth of lettuce seedlings, resulting in lettuce buds and root growth[16]. Furthermore, the CO_2 compensation point as one of the parameters reflected different environments and the effects of plants themselves on photosynthesis[41], which was affected mostly by the ratio of NO_3^--N and NH_4^+-N[42]. The air CO_2 had complex effects on plant growth and photosynthetic activity with many factors, such as the efficiency of the carbon fixation pathway[43]. Therefore, CO_2 management (e.g. CO_2 fertilization) was believed as a common technique in greenhouse vegetable cultivation. The increased air CO_2 could improve *Pn* of crops and leaf water content, reduce leaf *gs* and then stimulate plant growth[44]. Green vegetables had a strong enrichment effect on CO_2, which was absorbed through leaf photosynthesis to be transported to other tissues and then accumulated[45]. The enriching CO_2 to some extent could increase plant leaf area to improve effective leaf photosynthesis[46]. However, elevated CO_2 was also believed to decrease the rate of photosynthesis, stomatal conductance and internal CO_2 concentration of Chinese cabbages[23]. Nowadays, a certain photosynthetic model is helpful in determining the optimal CO_2 level required for crop production and calculate the reduced CO_2 demand during the whole planting period in greenhouses, maximizing crop photosynthesis and establishing the accurate CO_2 enrichment standard to determine the best environmental conditions[47,48].

In this paper, the results (Figure 2 and Figure 3) showed values of *Pn*, *gs*, *Ci*, *Tr* and *LWUE* significantly differed among all treatments. The treatment C1N4 got the highest *Pn* and *Ci* values at 3.05 μmol $CO_2/m^2 \cdot s$ and 352.8 μmol $CO_2/m^2 \cdot s$, respectively. The C1N2 received the biggest *gs* and *Tr* values at 0.70 mmol $H_2O/m^2 \cdot s$, and 2.31 mmol $H_2O/m^2 \cdot s$, respectively. But C1N2 received the lowest *Pn* and *LWUE* values at 2.42 μmol $CO_2/m^2 \cdot s$ and 1.05, while C0N2 had the minimum *gs* (0.47 mmol $H_2O/m^2 \cdot s$) and *Tr* (1.78 mmol $H_2O/m^2 \cdot s$). C0N0 got the lowest *Ci*. C0N2 and C1N4 had the largest LWUEs at 1.41 and 1.40. Briefly, although C1N4 might have the potential for leaf organic matter production, its yield had the minimum value under C1, but was bigger than C0N4. The reason might be that adding CO_2 fertilizer influenced the CO_2 absorbed in leaves, while the higher N in solution decreased root growth (as shown in Figure 1). The improved *Pn* also could be attributed to the changing air CO_2 concentration in the whole growth period, which showed that C1 treatment must have a higher maximum and average air CO_2 concentration. It was consistent with some research results[22]. It reported the maximum net CO_2 absorption, *Pn* and *gs* in some *Doritaenopsis* orchids growing below 1600 μmol/mol were higher than those growing at 450 μmol/mol, CO_2 concentrations between 800 and 1600 μmol/mol accelerated leaf initiation and net CO_2 absorption. Meanwhile, the increased leaf growth could also lead to the increased photosynthesis of individual plant[46].

Pearson correlation analysis results (Figure 5) displayed that both *Ci* and *LWUE* had no obvious correlation with other indexes (p>0.05). However, *Pn* had an extremely evident correlation with *LWUE* (p<0.01). The *gs* got an extremely evident correlation with *Tr*, and an extremely negative evident correlation with *LWUE* (p<0.01). *Tr* had an extremely negative obvious correlation with *LWUE* (p<0.01). The *LWUE* value was expressed as an index of crop water absorption and utilization efficiency, while the reduced leaf *Tr* could increase *LWUE*, maintain high carboxylation efficiency, and then increase the *Pn* of pepper seedlings[17]. Additionally, Chinese cabbage was more sensitive to high temperature, and stomatal closure above 25°C was not related to *Pn*[49]. Results of the variance analysis (Table 3) also demonstrated N, CO_2 and their N×CO_2 had extremely significant effects on *Pn* (p<0.01). Only N×CO_2 interaction had an extremely significant effect on *gs* (p<0.01). N and N×CO_2 interaction had significant effects on *Ci* (p<0.05), while CO_2 had an extremely significant effect (p<0.01). N had a significant effect on Tr (p<0.05), on which CO_2 and N×CO_2 interaction had extremely significant effects (p<0.01). Only N×CO_2 interaction showed an extremely significant effect on *LWUE* (p<0.01). Different concentrations (different salinity) of hydroponic solution could not change *Pn*[37].

Table 3 Conditions of photosynthesis indexes under N×CO_2 treatments (*n*=24)

Factor	*Pn*	*gs*	*Ci*	*Tr*	*LWUE*	*OPC*
N	**	ns	*	*	ns	ns
CO_2	**	ns	**	**	ns	ns
N×CO_2	**	**	*	**	**	ns

Note: ** and * displayed extremely significant differences at 0.01 and 0.05 levels, respectively. The ns displayed no significant difference.

4.3 Leaf anthocyanin

Anthocyanin (*OPC*) in leaf, fruit and other organs of crops is believed to improve physical health. Therefore, *OPC* became widely used in some fields[28], resulting in promoting purple leafy vegetable growth. Anthocyanin accumulation could be induced by light, low temperature, nutrient stress (e.g. nitrogen limitation), and air CO_2[29,30]. The *OPC* in grapefruit was reduced under air CO_2 management[30]. The leaf injury index was correlated with the number of anthocyanins[33]. In this study, the results of variance analysis displayed that there was no significant effect of solution N, CO_2 fertilizer or N×CO_2 on *OPC* (p>0.05, Table 3), respectively. However, *OPC* increased as N concentration in HNS went up under C0 (Figure 4). And *OPC* increased at first, and then decreased as N concentration increased under C1. Among all treatments, C1N2 significantly had the largest *OPC* at 1.35 mg/kg, while C1N0 received the lowest value. Surprisingly, yield and anthocyanin content showed an opposite changing law among different treatments. But, these results of purple cabbage were almost consistent with hydroponic purple lettuce in the former research[28]. Pearson correlation analysis results showed RL had extremely significant negative correlations with *OPC* (p<0.01, Figure 5), which might conform to long root might decrease *OPC* formation. Additionally, it also might be that other environmental factors (e.g., light and temperature) could also have obvious interaction effects on anthocyanin synthesis and coloration[50,51]. Therefore, further N×CO_2 effects on *OPC* formation needed to be conducted.

5 Conclusions

In this study, interaction effects of nitrogen (N) in hydroponic culture and air carbon dioxide (CO_2) fertilizer (N×CO_2) on growth, photosynthesis, yield and anthocyanin of a purple cabbage were preliminarily investigated. It was observed that N×CO_2 had extremely significant effects on leaf photosynthesis indexes. Meanwhile, N, CO_2 and their interaction had extremely significant effects on yield. The Pearson correlation analysis showed root and plant growth directly resulted in a higher output under N and CO_2 interaction. Notably, excessive root length might decrease anthocyanin content. Among all treatments, C1N2 (i.e., the interaction between CO_2 fertilizer and 0.2 g/L exogenous urea-N to hydroponic solution dilution) is optimally adapted as a coupling strategy to improve anthocyanin content and yield of hydroponic purple cabbage *Brassica campestris ssp.* chinensis.

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