INTERACTIVE EFFECTS OF ELEVATED CO_2 CONCENTRATION AND NITROGEN FERTILIZER APPLICATION ON NITROGEN DISTRIBUTION IN A COTTON–SOIL SYSTEM

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Abstract. This study aimed to investigate the beneficial effects of elevated CO_2 levels and nitrogen fertilization on the nitrogen distribution in a cotton-soil system in China, using CO_2 obtained as an industrial byproduct. A semi-open-top artificial climate chamber was used to investigate the effects of ambient CO_2 concentration (360 $\mu mol \cdot mol^{-1}$, Xinjiang) and elevated CO_2 concentrations (540 and 720 $\mu mol \cdot mol^{-1}$) and application of nitrogen (N) fertilizer (0, 150, 300, and 450 $kg \cdot hm^{-2}$) on cotton growth and available N distribution in the cotton–soil system. The results showed that the cotton biomass was positively influenced by the increase in CO_2 concentration and N application. The total N content in the cotton significantly increased with N application, and this increase was more significant with $CO_{2\,540}$ treatment compared with $CO_{2\,720}$ treatment. The N accumulation in buds and bolls was the highest, followed by leaves and then stems; it was the lowest in the roots. When the CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$, the soil NO_3^-–N content decreased significantly. The soil NH_4^+–N content slightly increased with 0 and 150 $kg \cdot hm^{-2}$ N application. When the CO_2 concentration was elevated to 720 $\mu mol \cdot mol^{-1}$, the soil NO_3^-–N content still decreased and the soil NH_4^+–N content increased. Overall, when the CO_2 concentration was elevated, the application of 300 $kg \cdot hm^{-2}$ N fertilizer significantly increased the cotton biomass and the total N content. It also promoted the absorption of soil N, especially that of NO_3^-–N. The findings provided practical guides for N application in the context of elevated CO_2 concentration in cotton fields.

Keywords: *elevated CO_2 concentration, nitrogen fertilizer application, cotton growth, biomass, plant total N content, soil NO_3^-–N, soil NH_4^+–N*

Introduction

The massive emission of industrial CO_2 has become promoting global warming (Guo et al., 2013). It is expected that the atmospheric CO_2 concentration will approach 1100 $\mu mol \cdot mol^{-1}$ (ppm) by the end of the 21st century (IPCC, 2014). Doubling of the concentration of CO_2 in the atmosphere affects not only climate, but also the agroecological environment (Ainsworth et al., 2005; Kimbal et al., 2016). Current climate projections indicate that the atmosphere on the earth's surface fluctuating more

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frequently, and extreme weather such as hyperthermia, drought and extreme cold will appear more frequently (NOAA-ESRL, 2018). The industrial emission CO_2 storage technology is becoming more and more mature, but the rational reuse method and the related utilization quantity are still in the bottle-neck period. The reuse of industrial emission CO_2 has been the focus of climate and environment research groups (Liu et al., 2018).

CO_2 is an important raw material for plant photosynthesis. It has been proven that a certain amount of CO_2 supplied could increase the biomass and economic yield of C_3 and C_4 plants (Morgan et al., 2001; Leakey et al., 2009). N is an important component of plant proteins and chlorophyll. Thus, the N addition has both direct and indirect effects on photosynthesis, respiration, and other metabolism pathways, affecting the distribution of N nutrients in plant-soil system. Studies have found that the photosynthetic capacity and plant biomass significantly increase under elevated CO_2 and N additions (Fang et al., 2000; Xu et al., 2003; Leakey et al., 2011; José et al., 2016); this in turn promotes the absorption and metabolism of N in plants (Bloom et al., 2002; Mitchell et al., 2018). Research by Pitelka (1994) showed that an elevated CO_2 concentration was conducive to the accumulation of carbohydrates in plant tissues, thus reducing the N content of plants. Recently Other studies (Talbot et al., 2000; Kimball et al., 2001; Dong et al., 2002; Lyu et al., 2015; Cai et al., 2016; Broughton et al., 2017a) have showed that C/N values in cotton, wheat, corn, and rice increased to different degrees under doubled CO_2 concentration, with a decrease in N content in the roots of leguminous plants, but an increase in the N content of the aboveground parts (Yang, 2002; Fitzgerald et al., 2005). It might be that legumes utilized rhizobia to fix N from the air to supplement their N needs to support their rapid growth under high CO_2 concentration. Xu et al. (2004) reported that CO_2 doubling reduced the N content of *Caragana korshinskii* and *Hedysarum laeve* leaves by 10.4% and 5.06%, respectively. However, in different ecosystems, the distribution and absorption of N in different parts of the plant under high CO_2 concentration is related to the photosynthetic pathways of the plant itself, the supply of exogenous N nutrients, the nutrient status of the soil itself, and the amount of N absorbed (Oberbauer et al., 1986; Grunzweig et al., 2001; Charles et al., 2019). Therefore, following increases in CO_2 concentration, exogenous N nutrition could compensate for increased plant growth and metabolism (Stitt et al., 1999; Johnson, 2000; Yang, 2002; Zhang, 2002). It is known that a shortage of N limits the production of CO_2 (Larigauderie et al., 1998; Joel et al., 2001). Under low N conditions, the photosynthetic rates under high CO_2 concentration is lower than under normal CO_2 concentration. Further research revealed that increasing the availability and simultaneous supply of N source can prevent this photosynthetic adaptation (Guo et al., 2006; Mitchell, 2018).

There has been a significant focus of research on the response and adaptation of plants in different ecosystems under elevated CO_2 concentration and different N application and the research objects mainly focuse on horticultural plants, rice, wheat and forest. Cotton is a typical C_3 plant, and empirical and modeling studies (Morgan et al., 2007; Zhang et al., 2017; Broughton et al., 2017b; Li et al., 2020) have shown that possible increase in sensitivity of the physiology and plant productivity to enhancing atmospheric CO_2 concentration. These researches focused on the effect of CO_2 enrichment on cotton growth while the interaction mechanism of CO_2 and N coupling on cotton ecosystem is not clear. Xinjiang is a major agricultural province and is the biggest commercial cotton production base in China. Cotton plays an irreplaceable role in agricultural production and economy in China and Xinjiang. The latest statistical data (data source: National Climatic Data Center in China) and the current studies (Gao et al., 2015; Cui et al., 2019) have shown that the ambient CO_2 concentration in background air was generally about ~330-372 ppm, which is far from meeting the photosynthetic demand of cotton, and elevated CO_2 concentration can improve the light saturation point of cotton.

The objective of this study focused on the impact of elevated CO_2 and N fertilizer application in the Xinjiang cotton field ecosystem. The project used a semi-open top artificial climate chamber to investigate the response of cotton plants to increases in CO_2 at different levels of N fertilizer application. The total N content of cotton plant and the distribution of NO_3^--N and NH_4^+-N in rhizosphere soil were determined to reveal the response mechanisms of cotton and the soil to the CO_2 and N interaction. Given that the response mechanism of cotton is used to determine the optimal N application level under elevated CO_2 concentrations in cotton canopy, our results would be beneficial to guide the optimal application of N fertilizers in cotton fields under the high CO_2 concentration. Meanwhile, the findings of this study provided a technical scheme for the rational utilization of industrial CO_2 emission. It also provided a scientific basis for accurately predicting the terrestrial ecosystem response and crops yield potential model in the context of elevated CO_2 concentration, and simultaneously it will be of great significance to the healthy and sustainable development of China's cotton industry.

Materials and experimental methods

Research area

The experiment was carried out in 2016-2018 in a semi-open-top artificial climate chamber at the Xinjiang Academy of Agricultural Sciences in China (N44°18'.288, E85°59.961'). Our previous studies (Yin et al., 2011; Gao et al., 2015; Yin et al., 2016) have found that the CO_2 concentration at surface boundary layer in cotton field was generally about ~330-360 ppm from 10 a.m. to 6 p.m. Xinjiang is a typical continental arid climate, the annual precipitation in this area is ~125.0-207.7 mm, the annual evaporation is 1946 mm, the annual average temperature is ~7.5-8.2 °C, the annual sunshine hours are 2526-2874 h, with the sunshine hours in the growing season being ~1900-2000 h, and the total radiation of light per year is next to that of Qinghai-Tibet Plateau. The frost-free period is about ~160 days, and the accumulated temperature of ≥ 10 °C is ~3570-3729 °C. The basic physical and chemical properties of the soil used in the study are shown in *Table 1*. Cotton was planted on April 15 and harvested on September 30.

Table 1. *Physical and chemical properties of the soil used in the study*

Soil type	**Soil texture**	**Soil layer (cm)**	**Organic matter ($g \cdot kg^{-1}$)**	**Alkaline hydrolysis nitrogen ($mg \cdot kg^{-1}$)**	**Available P ($g \cdot kg^{-1}$)**	**Available K ($g \cdot kg^{-1}$)**	**pH**
Gray desert soil	Medium loam	0-20	6.94	41	21	99	8.3
		20-40	5.73	33	14	103	8.1

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Test materials

The test crop was cotton and the variety was Xinluzao 33, the density was 22.5 millon plant per ha; the fertilizer used was urea ($CO(NH_2)_2$), with a measured N content of 46%; CO_2 was sourced from a gas cylinder supplied by the Shihezi Tiangang acetylene plant.

Test device

The experimental set up a semi-closed open top artificial climate chamber, surrounded by a light-transmissive plastic film (blue film in a greenhouse), with a film height of 1.5 m (*Fig. 1A*). The outside of the gas chamber was connected to a CO_2 gas cylinder and CO_2 was administered via drip irrigation belt that ran inside the chamber (*Fig. 1B*). When the cotton entered the flowering period, which was the most vigorous stage of cotton growing, CO_2 was released from 12:00 to 15:00 (the illumination is strongest in Xinjiang) daily via the drip irrigation belt. The gas input was controlled by a CO_2 decompression flow valve, and the internal concentration was measured in real time through a portable infrared CO_2 concentration detector (AT-B-CO_2, Beijing Antai Jihua Technology Co., Ltd.) with S-type distribution.

A B

Figure 1. *(A) Semi-closed open top artificial climate chamber. (B) CO_2 gas cylinder and pressure reducing valve*

Experimental design

The test was carried out in a split zone design. Three CO_2 concentration levels were used: 360 μmol·mol^{-1} (CK, Xinjiang background level), 540 μmol·mol^{-1} (1.5 times higher than the background level) and 720 μmol·mol^{-1} (two times higher than the background level) (referred to as $CO_{2\,360}$, $CO_{2\,540}$ and $CO_{2\,720}$, respectively). Four levels of application N (0, 150, 300, and 450 kg·hm^{-2}; referred to as N_0, N_{150}, N_{300}, and N_{450}, respectively) were used for each CO_2 concentration based on the results of previous studies and local fertilization levels. The moderate water and fertilizer conditions in the northern cotton area of Xinjiang required 300 kg of pure nitrogen to determine the N concentration gradient for this study (Yin et al., 2010, 2011). The CO_2 concentration was the main treatment, and the N fertilizer was the secondary treatment. There were 12 treatments, each repeated three times, over a total of 36 plots. Each the plot area was 42 m^2 (2.8 m × 15 m). The main treatment plots were separated by median intervals and whereas the secondary treatment plots were next to each other.

N fertilizer was applied in the form of analytical grade urea, and the phosphorus and potassium fertilizers were applied as KH_2PO_4 ($K_2O \geqslant 33.9\%$, $P_2O_5 \geq 51.5\%$). The fertilization measures were based on the local field requirements: thus, 30% N fertilizer was applied initially, followed by 40% at the first time of watering and 30% at the second time of watering.

The phosphate fertilizer (P_2O_5) dosage was 125 $kg \cdot hm^{-2}$, potassium fertilizer (K_2O) dosage was 54 $kg \cdot hm^{-2}$, and both were used as base fertilizers. The N fertilizer was applied according to the proportion of base application 30%, application 40% for the first irrigation and application 30% at the second water drip, respectively. The times of irrigation was nine in the whole growth period. Treatments with increased CO_2 concentrations began at the start of the cotton flowering period (July 18), under the conditions of optimal light intensity (from ~12:00 h to 15:00 h). The drip irrigation capillary system was used to inject CO_2 gas into the chamber until the desired CO_2 concentration was reached. N fertilizer was applied simultaneously with the water droplets to achieve the simultaneous application of carbon and N. The other field management measures (e.g., *verticillium* wilt control, topping and so on) were the same as used generally in cotton fields.

Test indicators and analysis methods

On 18 August (after 30 days of CO_2 gas addition), three points were selected from each treatment plot according to Z pattern and the size of each point was 2.8 m × 2 m. Ten healthy cotton plants were selected from each point and divided into their aboveground and belowground tissues, which were then heated in an over at 105 °C for 30 min and then dried at 80 °C for 24 h. The dry weight was then determined. After smashing and sieving, 10 mg of the plant sample was measured by using the standard Kjeldahl method. In each cotton plant sampling point, it was used that tubular auger (the length of auger is 1 m, bit depth is 20 cm and diameter is 3 cm) to collect fresh soil at the depth of ~0–20 cm and ~20–40 cm soil layer; 1 $mol \cdot L^{-1}$ KCl 50 mL was added to 10 g of the fresh soil sample and then shaken for 30 min. The suspension was then filtered through filter paper, and the nitrate nitrogen (NO_3^-–N) and ammonium nitrogen (NH_4^+–N) in the soil were using a Continuous Flow Analytical System (CFA). Soil moisture was determined by the drying method.

SPSS 19.0 was used to analyze the data using ANOVA and P value test significance tests. The functional relationship between CO_2 concentration and N application level was constructed by using Sigmaplot 12.5 and Excel 2007 to analyze the gradient changes in total N and soil NH_4^+–N and NO_3^-–N content. The datas in chart were the anverage of 2016–2018 year.

Results and analysis

Effects of elevated CO_2 concentration and N fertilizer application on cotton biomass

In this study, dry matter weight was adopted to analyse the changes in cotton biomass. ANOVA showed that the biomass of cotton organs and the whole plant was significantly affected by CO_2 concentration, N application and the interaction of CO_2 and N (*Table 2*). In general, the biomass of buds and bolls, stems, and the whole plant was increased significantly with CO_2 concentration elevation, whereas the biomass of leaves and roots in $CO_{2\ 540}$ concentration was higher than that in $CO_{2\ 720}$ concentration.

Gao and Guo (2003) reported that elevated CO_2 concentrations could also promote the biomass increase in desert plant, and the roots, stems, and leaves responded differently to elevated CO_2 concentrations. Some studies indicated that the initially promotion effect high concentration CO_2 on plant would gradually disappear as time going by (Chen, 2005). At the same CO_2 concentration, the biomass of different cotton organs increased with N fertilizer application increasing, with more significant increases occurring at the higher N application levels, which was consistent with the results of some researchers (Zhang et al., 1999; Stitt et al., 1999). Overall, at the flowering and boll-forming stages, the biomass of buds and bolls was the highest, followed by leaves, and lowest in stems and roots, indicating the transport of nutrients from the roots to reproductive organs.

The results also showed that the effect of elevated CO_2 concentrations on cotton biomass was closely related to the supply of mineral nutrients. When atmospheric CO_2 concentrations elevated from 350 $\mu mol \cdot mol^{-1}$ to 700 $\mu mol \cdot mol^{-1}$, the crop yield and biomass could increase by 24–25% (Kimball, 2016), but this response would be lower in the case of water and nutrients deficiency (Oechel, 1994). Therefore, when considering the growth–promoting effects of elevated CO_2 concentrations on plant growth, it is necessary to ensure the appropriate timing of fertilizer application (Roser et al., 1999; Yang et al., 2007; Coskun et al., 2016; Zhang et al., 2017).

Table 2. *Changes in the biomass of cotton in response to elevated atmospheric CO_2 and N application (g)*

CO_2 concentration ($\mu mol \cdot mol^{-1}$)	**N fertilizer application ($mg \cdot kg^{-1}$)**	**Cotton organ**				
		Leaf	**Stem**	**Bud and boll**	**Root**	**Total plant**
$CO_{2\ 360}$	N_0	11.64±0.09 d	7.23±0.09 d	16.77±0.07 cd	4.07±0.16 c	39.68±0.23 d
	N_{150}	13.66±0.46 c	8.22±0.10 c	17.77±0.11 c	4.83±0.06 b	44.48±0.39 c
	N_{300}	14.29±0.15 b	9.10±0.09 b	18.85±0.05 b	5.09±0.07 ab	47.33±0.24 ab
	N_{450}	16.36±0.08 a	10.23±0.07 a	20.11±0.12 a	5.70±0.05 a	51.34±0.25 a
	Average	13.99±1.87	8.69±0.16	18.38±1.47	4.92±0.65	45.96±4.98
$CO_{2\ 540}$	N_0	12.27±0.15 d	8.41±0.15 d	16.86±0.07 d	4.94±0.07 d	42.48±0.05 d
	N_{150}	13.78±0.09 c	9.96±0.03 c	18.24±0.31 c	5.38±0.05 c	47.36±0.38 c
	N_{300}	15.91±0.14 b	10.22±0.08 b	20.42±0.57 b	6.19±0.05 ab	52.74±0.51 b
	N_{450}	17.38±0.12 a	11.17±0.08 a	22.11±0.22 a	6.56±0.09 a	57.22±0.43 a
	Average	14.83±2.10	9.94±1.04	19.41±2.49	5.77±0.70	49.95±6.07
$CO_{2\ 720}$	N_0	12.02±0.05 d	9.13±0.04 c	17.15±0.10 d	4.95±0.09 b	43.30±0.06 d
	N_{150}	13.75±0.06 c	10.96±0.06 b	19.98±0.04 c	5.18±0.10 b	49.87±0.14 c
	N_{300}	14.42±0.16 b	11.35±0.09 a	22.22±0.59 b	5.58±0.15 ab	53.57±0.64 b
	N_{450}	16.69±0.17 a	11.75±0.48 a	23.71±0.21 a	5.99±0.05 a	58.14±0.80 a
	Average	14.23±2.17	10.79±1.07	20.77±2.38	5.43±0.42	51.22±6.14
Sources of variation						
CO_2		**	**	**	*	**
N		**	**	**	*	**
CO_2 * N		*	*	**	NS	**

Different letters in the same column indicated significant difference among treatments at 0.05 level. ** indicated $P < 0.01$, * indicated $P < 0.05$, *NS* indicated no significant difference at 0.05 level

Effects of elevated CO2 concentration and N fertilizer application on the total N content in different organs of cotton

The changes in total N content of leaves are shown in *Figure 2A*. Leaves are important photosynthetic organs for photosynthesis and the major source for carbohydrates. Elevated CO_2 concentrations are hypothesized to be beneficial to photosynthesis and respiration in leaves (Oberbauer et al., 1986; Wang et al., 2011; Zhang et al., 2016), which is also likely to affect nutrient absorption by the leaves. Analysis of variance showed that elevated CO_2 concentration, N fertilizer application and the interaction of CO_2 concentration and N fertilizer could significantly increase the total N content in leaves ($P = 0.005$, 0.000 and 0.002, respectively). In the same CO_2 concentration treatment, the total N content in leaves increased significantly with increasing N fertilizer application ($P = 0.012$). At the same N level, the total N content in leaves in the $CO_{2\ 720}$ treatment group was higher than that in the $CO_{2\ 540}$ treatment group, but there was no significant difference. Under high CO_2 concentrations, C_3 plants were able to adapt their photosynthesis under low N conditions (Zhou et al., 2006; Xia et al., 2019). These results indicated that when the CO_2 concentration was elevated to between 540 and 720 $\mu mol \cdot mol^{-1}$, it promoted the N absorption and utilization by leaves. The effect of elevating CO_2 concentration on the N demand resulting from increased growth was compensated by increasing N fertilizer application. Previous researches also showed that N nutrition delays the senescence of mature leaves and improves the adaptability of plant to adverse conditions (Zhang et al., 2002).

Changes in total N content in buds and bolls are shown in *Figure 2B*. This result showed that elevated CO_2 concentration and N fertilizer application had significant effects on the total N content in buds and bolls ($F = 19.13$ and 29.10, respectively), and this was also significant in terms of the interaction between CO_2 concentration and N fertilizer ($F = 2.89$). Under ambient CO_2 conditions, the total N content in buds and bolls showed a significant positive correlation with N fertilizer application dosages. When the CO_2 concentration was elevated by 0.5 and 1.0 times, the total N content in buds and bolls significantly increased, and this was more significant in the $CO_{2\ 540}$ treatment group. Compared with $CO_{2\ 360}$ treatment, when the CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$, the total N content in buds and bolls at N_0, N_{150}, N_{300}, and N_{450} level increased by 12.57%, 13.41%, 28.42% and 17.50% respectively; when the CO_2 concentration was elevated to 720 $\mu mol \cdot mol^{-1}$, the total N content in buds and bolls showed had no significant changes at N_0 and N_{150} level, but increased by 14.75% and 12.50% at N_{300} and N_{450} level, respectively. In general, of the different CO_2-N combination treatments, the total N content in buds and bolls was the highest in $CO_{2\ 540}$-N_{300} treatment group.

Changes in total N content in stems are shown in *Figure 2C*. The total N content in stems increased with the increasing of N fertilizer application in each CO_2 concentration treatment. Compared with ambient CO_2 concentration treatment group, the total N content in stems increased significantly when CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$ ($P = 0.703$), although there was no significant change when CO_2 concentration was elevated to 720 $\mu mol \cdot mol^{-1}$ ($P = 0.53$). This suggested that when the atmospheric CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$, N fertilizer application was more beneficial to promote the N nutrition increasing in stems.

Changes in total N content in roots are shown in *Figure 2D*. There were no significant differences in total N content in roots among the different CO_2 concentration treatments ($P = 0.051$). In ambient CO_2 concentration treatment, the total N content in roots increased with increasing N fertilizer application dosages ($P = 0.016$). When the CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$ and 720 $\mu mol \cdot mol^{-1}$, the interaction of CO_2 concentration and N fertilizer application had significant effect on N content in roots and the total N content at N_{300} was lower than that at N_{150} level.

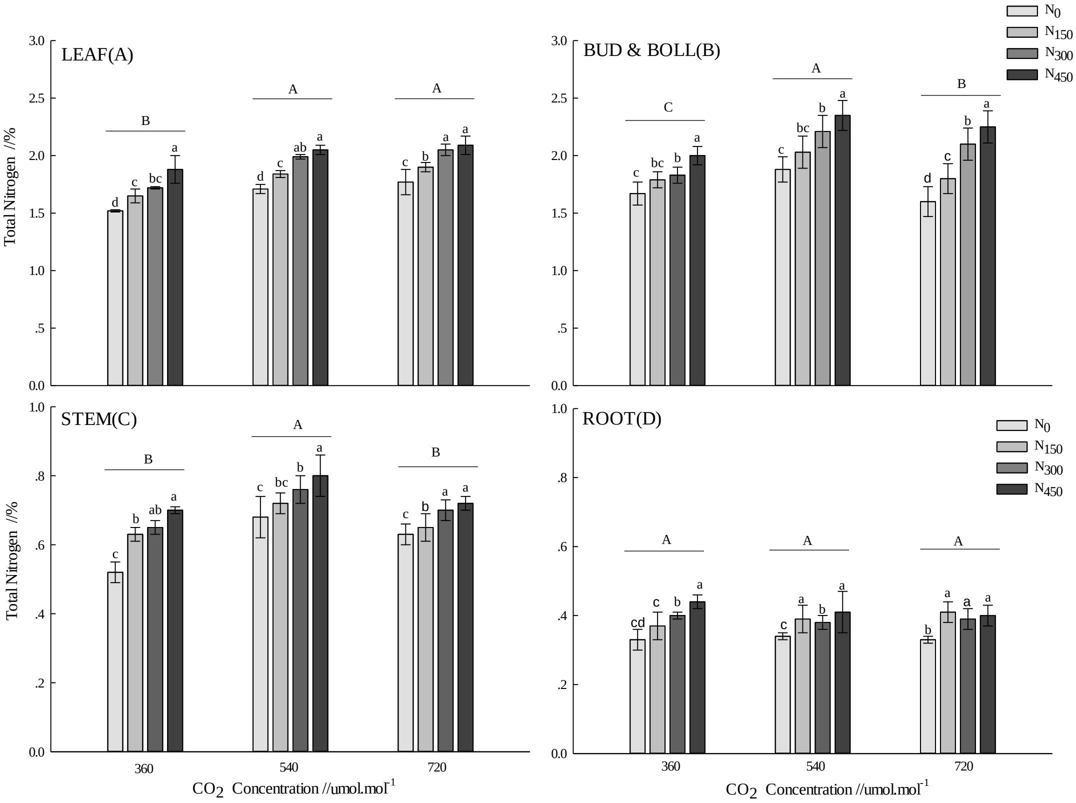

Figure 2. Effects of elevated CO_2 and N fertilizer application on the total N distribution in cotton leaf (A), bud and boll (B), stem (C), and root (D). Different lowercase letters in the column indicated significant difference among different N treatments at $P < 0.05$. Different capital letters in the column indicate significant difference among different CO_2 treatments at $p < 0.05$

In general, different combinations of CO_2 concentration and N fertilizer had significant effects on N absorption and utilization of different cotton organs. The total N content in buds and bolls was the highest, followed by leaves, then was in stems, being lowest was in roots. This was probably because the combination of elevated CO_2 concentrations and N fertilizer applications promoted the photosynthesis and growth metabolism of the aboveground tissues of cotton, which then needed to absorb nutrition from belowground tissues to support increase. Thus, N nutrients were transported to the buds, bolls and other vegetative organs of cotton via the roots and stems to meet the nutritional needs of reproductive growth (Hu et al., 2006; Wang et al., 2010; Yin et al., 2011).

The effects of elevated CO_2 concentration and N fertilizer application on total N in the whole cotton plant are shown in *Table 3*. When the CO_2 concentration was elevated by 0.5 times and 1.0 times, the total N content in whole cotton plant increased significantly compared with the ambient CO_2 concentration, this increasing effect was more significant in $CO_{2\ 540}$ treatment. That indicated the CO_2 concentration was elevated to 720 μmol·mol^{-1} and above would restrict N nutrient utilization by the cotton. Under the same CO_2 concentration treatment, the total N content in the whole plant increased with the N fertilizer application dosages, which was consistent with the general fertilization effect. Meantime, the interaction of elevated CO_2 concentration and N fertilizer application did have a significant effect on the total N content in the whole cotton plant ($P = 0.008$).

This suggests that when the atmospheric CO_2 concentration is elevated to ~540-720 μmol·mol^{-1}, which promotes the N nutrient absorption and accumulation in cotton plant, it would be necessary to increase the application of external N fertilizer to compensate for the nutrient absorption by the plants caused by the increasing CO_2 concentration.

Table 3. *The total N content in the whole cotton plant under different CO_2 and N treatments (%)*

Treatments	**N_0**	**N_{150}**	**N_{300}**	**N_{450}**
$CO_{2\ 360}$	4.11 ± 0.20 dC	4.63 ± 0.12 cC	4.82 ± 0.04 bB	5.08 ± 0.21 aC
$CO_{2\ 540}$	4.83 ± 0.17 dA	5.08 ± 0.12 cA	5.35 ± 0.40 bA	5.65 ± 0.17 aA
$CO_{2\ 720}$	4.56 ± 0.11 dB	4.97 ± 0.25 cAB	5.28 ± 0.06 abA	5.45 ± 0.23 aAB

The different lowercase letters in the same row indicated significant differences among different N treatments at $P < 0.05$. The same capital letters in the same column indicate no significant differences among different CO_2 treatments at $P < 0.05$

Effects of elevated CO_2 concentration and N fertilizer application on root soil NO_3^-–N content in cotton fields

The response of NO_3^-–N content to different CO_2 and N fertilizer treatments varied in the ~0–20 cm and ~20–40 cm soil layers, as shown in *Figure* 3*A* and *B*, respectively. Soil NO_3^-–N content decreased significantly with increasing CO_2 concentrations and this decrease was more significant at the $CO_{2\ 540}$ level than that at the $CO_{2\ 720}$ level. The interaction of elevated CO_2 concentration and N fertilizer application had a significant effect on the NO_3^-–N content in the ~0–20 cm soil layer and ~20–40 cm soil layer ($P = 0.032$ and 0.000, respectively).

In ambient CO_2 concentration, the NO_3^-–N content in ~0–20 cm soil layer significantly increased with increasing N fertilizer application ($P = 0.001$). When the CO_2 concentration was elevated to 540 μmol·mol^{-1}, the soil NO_3^-–N content showed no significant difference between different N application dosages ($P = 0.420$), although it was lowest at the N_{300} level. When the CO_2 concentration was elevated to 720 μmol·mol^{-1}, the soil NO_3^-–N content increased with the increase in N fertilizer application ($P = 0.040$).

At the same N fertilizer application level, the soil NO_3^-–N content in root layer decreased significantly in proportion to the elevated CO_2 concentrations. Compared with the ambient CO_2 concentration, in the $CO_{2\ 540}$ concentration treatment group, the soil NO_3^-–N content decreased by 25.90%, 25.05%, 47.77% and 37.38% at N_0, N_{150}, N_{300}, and N_{450} level, respectively; in the $CO_{2\ 720}$ concentration treatment group, the soil NO_3^-–N content decreased by 6.94%, 4.52%, 18.31% and 19.30% at N_0, N_{150}, N_{300} and N_{450} level, respectively. The NO_3^-–N content at the N_{300} and N_{450} levels was significantly lower than at N_0 and N_{150} level under elevated CO_2 concentrations. In a general, in the interaction treatment groups of CO_2 concentration and N fertilizer, the soil NO_3^-–N content was lowest in CO_2 $_{540}$–N_{300} treatment group. Other studies reported that, at high N application levels, the soil NO_3^-–N content decreased significantly under elevated CO_2 concentrations and N fertilizer application, mainly due to N application significantly promoted the growth and metabolism of the plants (Mauney et al., 1994; Ma et al., 2004; Liu et al., 2018), increasing NO_3^-–N absorption by plants from soil, thus affecting the soil N nutrients conditions. In addition, an anaerobic environment was formed by the drip irrigation system, inhibiting soil-nitrifying microbial activity and reducing the soil NO_3^-–N content. The reduction in the soil NO_3^-–N content in the CO_2 $_{540}$ concentration treatment was greater than that in the CO_2 $_{720}$ concentration treatment; this suggests that the level of CO_2 enrichment was too high. If this negatively impacted the ability of cotton to adapt to higher levels of CO_2 then this would inhibit the absorption of soil nutrients by the plants.

The soil NO_3^-–N content decreased with the soil depth increasing. In the ~20–40 cm soil layer, the changes in NO_3^-–N content were the same as in the ~0–20 cm soil layer among different CO_2 concentrations and N fertilizer treatments; however, the increasing trends in NO_3^-–N content were more significant in the ~0–20 cm soil layer, given the interaction effects between the increasing CO_2 concentration and N fertilizer application.

Effects of elevated CO_2 concentration and N fertilizer application on root soil NH_4^+–N content in cotton fields

The soil NH_4^+–N content varied significantly under different CO_2 concentration and N fertilizer treatments as shown in *Figure* 3*C* and *D*. ANOVA analysis showed that elevated CO_2 concentration, N fertilizer application and the interaction of CO_2 and N all had significant effects on NH_4^+–N content in the ~0–20 cm soil layer (F = 176.7, 98.06, and 9.925, respectively) and in the ~20–40 cm soil layer (F = 191.784, 65.150, and 6.270, respectively).

In the ~0–20 cm soil layer, in the ambient CO_2 concentration, the NH_4^+–N content significantly increased with increasing N fertilizer addition ($P = 0.000$), and this was more significant at the N_{300} and N_{450} levels. Compared with the ambient CO_2 concentration, the NH_4^+–N content showed a slightly increase at the N_0 and N_{150} levels when the CO_2 concentration was elevated to 540 $\mu mol \cdot mol^{-1}$, but a decrease at the N_{300} and N_{450} levels (reduced by 16.40% and 17.94%, respectively). This suggests that the interaction of CO_2 concentration and the high N fertilizer application could significant promote the NH_4^+–N nutrition absorption by cotton plants. This was consistent with the trends also recorded in the total N content in cotton plants (*Table 3*). When the CO_2 concentration was elevated to 720 $\mu mol \cdot mol^{-1}$, the soil NH_4^+–N content was significantly increased with increasing N fertilizer application. This was consistent with previous studies on other plants, such as wheat, *Phoebe bournei* and rice (Bloom et al., 2002; Han et al., 2003; Liu et al., 2018). In rice–wheat rotation farmland ecosystems, following an increase in CO_2 concentration, soil ammonia-oxidizing bacteria decreased, as did the nitrification activity of the dominant strain, whereas the soil NH_4^+–N mass fraction increased (Lin et al., 2005). This trend was similar to the soil NO_3^-–N content; overall, the soil $NH4^+$–N content in the CO_2 $_{540}$–N_{150} and CO_2 $_{540}$–N_{300} treatment groups was lower than that in other CO_2–N treatment groups.

Compared with the ~0–20 cm soil layer, the NH_4^+–N content in the ~20–40 cm soil increased, but not significantly. The soil NH_4^+–N content increased with increasing CO_2 concentration in the ~20–40 cm soil layer ($P = 0.000$). When the CO_2 concentration was elevated, the soil NH_4^+–N content was increased in proportion to the N application dosage.

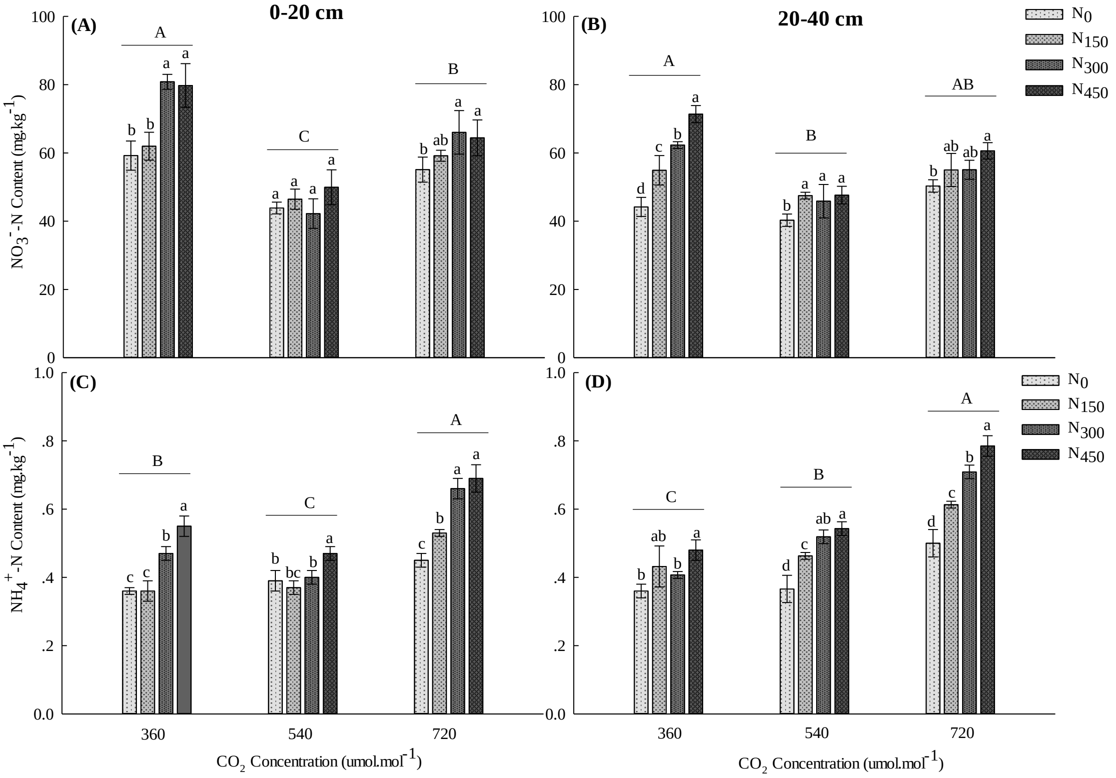

Figure 3. *Effects of elevated CO_2 concentration and nitrogen fertilizer application on NO_3^-–N content (A, B) and NH_4^+–N content (C, D) in cotton field soil. Different lowercase letters in the column indicated significant differences among different N treatments at $P < 0.05$. Different capital letters in the column indicate significant differences among different CO_2 treatments at $P < 0.05$*

Discussion

Studies have shown that elevated CO_2 concentration could promote the growth and N absorption of plants (Dijkstra et al., 2010; Xiao et al., 2017). Under the condition of enhancing CO_2 concentration, photosynthetic adaptation of C_3 plants mainly occurs under the condition of low N conditions (Stitt et al.,1999; Wang et al., 2015). Further research demonstrated that improvement in the availability of N sources and synchronous supply capacity can prevent the occurrence of adaptation (Mitchell et al., 2018).

The effect of elevated atmospheric CO_2 concentration on N uptake by plants is related to atmospheric CO_2 concentration, plant species and the form of absorbed N as well as other factors. Studies have demonstrated that cotton, wheat, corn, rice and other crops have various degrees of C/N elevation in the body under the high concentration of CO_2 and the N content of plants decreased (Lee et al., 2013), while, there was no change in legumes (Dong et al., 2002; Wang et al., 2010). This may be attributed to the fact that legume plants have *rhizobia*, which can fix N element from the air and supplement the requirement of N nutrition for rapid growth of plants under high CO_2 concentration rather than the absence of such functions in non-leguminous plants.

Previous research reported that when the CO_2 concentration was elevated to ~500–700 μmol·mol^{-1}, higher N fertilizer application promoted the N nutrition absorption and transformation by plants (Xu, 2012). In this study, there was also significant effect on cotton growth and N absorption when CO_2 concentration enrichment and N fertilizer application. As the atmospheric CO_2 concentration enriched, the biomass and N nutrition accumulation in the cotton increased, whereas the available N content in soil was decreased; this trend was most significant with the high N fertilizer application under a CO_2 $_{540}$ concentration. Deiglmayr et al. (2004) had found that soil N availability was reduced by elevated CO_2, the increase in plant growth may only be possible when plants increase their available nitrogen uptake. Analyzing the correlation between the changes in N content in cotton and soil under different CO_2–N combination treatments showed that high N fertilizer application was beneficial to the absorption and utilization of soil N in cotton field, especially NO_3^-–N. Changes in the N content of soil are related to the forms of soil inorganic N in different regions. For example, the soil N in northern China mainly occurs in the form of NO_3^-–N; thus, cotton grown in northern regions shows the selective absorption of NO_3^-–N (Hu et al., 2006; Liu, 2010). In addition, an anaerobic environment is formed by coated cultivation, and hyperthermia and drought environments are caused by CO_2 concentrations enrichment, which will affect the activity of nitrifying microorganisms in farmland soil, which could also reduce soil the NO_3^-–N content (Chen et al., 2002; Wang et al., 2010).

Conclusions

The results showed that the increase in cotton growth was positively influenced by the increase in N application with increasing CO_2 concentration, which was most significant at CO_2 $_{540}$ concentration treatment. During the flowering and boll-forming stages, the biomass was highest in the buds, followed by leaves, stems, and roots. When the CO_2 concentration was elevated, the total N content in the cotton plant was significant increased with the N fertilizer application increasing, and this increase effect was more significant in the CO_2 $_{540}$ treatment than that in CO_2 $_{720}$ treatment. The total N accumulation content in buds and bolls was the highest, followed by leaves and then stems, being lowest in the roots. The changes in soil NO_3^-–N and NH_4^+–N content were also significant with the interaction of CO_2 concentration enrichment and N fertilizer application. When the CO_2 concentration was elevated to 540 μmol·mol^{-1}, the soil NO_3^-–N content decreased significantly, the soil NH_4^+–N content slightly increased with 0 and 150 kg·hm^{-2} N application. When the CO2 concentration was elevated to 720 μmol·mol^{-1}, the soil NO_3^-–N content still decreased and the soil NH_4^+–N content increased.

Overall, when CO_2 concentration was elevated to ~540–720 μmol·mol^{-1}, the application of 300 kg·hm^{-2} N fertilizer significantly increased the cotton biomass and the total N content. It also promoted the absorption of soil N, especially of NO_3^-–N. This suggests that when N fertilizer supplied effectively, the atmospheric CO_2 concentration enrichment could promote the photosynthesis and growth metabolism of cotton, which also increase the absorption and utilization of soil N nutrients by the plants. Thus, as literature suggests that the N fertilizer application to Xinjiang cotton fields in the future should be increased when atmospheric CO_2 concentration enrichment. However, the impact of CO_2 concentration and N nutrients on cotton growth and available N utilization are governed by complicated physiological and ecological processes. Thus, the interaction of CO_2 concentration and N fertilizer requires further verification, as do the mechanisms involved in the impacts of elevated CO_2 concentration on different forms of N transformation and utilization in cotton–cropping systems.

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