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Effect of nitrogen application and elevated CO_2 on photosynthetic gas exchange and electron transport in wheat leaves

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Abstract

Nitrogen (N) availability is a critical factor affecting photosynthetic acclimation of C_3 plants under elevated atmospheric CO_2 concentration ($[CO_2]_e$). However, current understanding of N effects on photosynthetic electron transport rate and partitioning, as well as its impact on photosynthesis under $[CO_2]_e$, is inadequate. Using controlled environment open-top chambers, wheat (*Triticum aestivum* L.) was grown at two N levels (0 and 200 mg(N) kg^{-1} soil) and two atmospheric CO_2 concentrations of 400 ($[CO_2]_a$) and 760 μmol mol^{-1}($[CO_2]_e$) during 2009 and 2010. Under $[CO_2]_e$ high N availability increased stomatal conductance and transpiration rate, reduced limitations on the activity of triose phosphate isomerase, a Calvin cycle enzyme, and increased the rate of net photosynthesis (P_N). Considering photosynthetic electron transport rate and partitioning aspects, we suggest that greater N availability increased P_N under $[CO_2]_e$ due to four following reasons: (*1*) higher N availability enhanced foliar N and chlorophyll concentrations, and the actual photochemical efficiency of photosystem (PS) II reaction centers under irradiance increased, (*2*) increase of total electron transport rate and proportion of open PSII reaction centers, (*3*) enhancement of the electron transport rate of the photochemical and carboxylation processes, and (*4*) reduced limitations of the Calvin cycle enzymes on the photosynthetic electron transport rate. Consequently, sufficient N improved light energy utilization in wheat flag leaves under $[CO_2]_e$, thus benefiting to photosynthetic assimilation.

Additional key words: CO_2 concentration; electron transport rate and partitioning; nitrogen availability.

Introduction

Photosynthetic acclimation is considered to be related to the reduction in leaf N content, chlorophyll (Chl) content, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content and activity, and the limitation of ribulose-1,5-bisphosphate (RuBP) regeneration under elevated atmospheric $[CO_2]$ (Rogers and Ellsworth 2002). Photosynthetic acclimation is generally greater, when N supply is deficient, and it can disappear, when N supply is adequate (Stitt and Krapp 1999, Isopp *et al.* 2000). Rogers *et al.* (1998) demonstrated that photosynthetic acclimation under $[CO_2]_e$ and low N was a result of low N, limiting the sink development rather than direct effects of N supply on photosynthesis. Conversely, other studies from the Free-Air CO_2 Enrichment (FACE) experiments suggested that the reduction in Rubisco content and activity was species-specific. Rubisco content and activity measured on a protein content basis did not decrease under $[CO_2]_e$ (Ainsworth and Long 2005). It was concluded that photosynthetic acclimation actually reflected the plant nutrition status instead of directly

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Abbreviations: C_i – intercellular CO_2 concentration; $[CO_2]$ – CO_2 concentration; $[CO_2]_a$ – ambient atmospheric CO_2 concentration; $[CO_2]_e$ – elevated atmospheric CO_2 concentration; Chl – chlorophyll; *E* – transpiration rate; F_v'/F_m' – maximal quantum yield of PSII under irradiance; g_s – stomatal conductance; HNAC – treatment of $[CO_2]_a$ + N200; HNEC – treatment of $[CO_2]_e$ + N200; J_c – electron transport partitioned to carboxylation; J_F – total electron transport rate of PSII reaction centers; J_o – electron transport partitioned to photorespiration; LNAC – treatment of $[CO_2]_a$ + N0; LNEC – treatment of $[CO_2]_e$ + N0; N0 – low N availability; N200 – high N availability; NPQ – nonphotochemical fluorescence quenching; P_N – rate of net photosynthesis; PNUE – photosynthetic nitrogen-use efficiency; PSII – photosystem II; q_P – photochemical fluorescence quenching coefficient; R_D – daytime dark respiration; R_L – respiration in the light; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; TPU – rate of triose phosphate utilization; V_c – Rubisco carboxylation rate; V_o – Rubisco oxygenation rate; WUE – leaf water-use efficiency; Φ_{PSII} – actual PSII efficiency under irradiance.

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affecting photosynthesis under $[CO_2]_e$ (Saxe *et al*. 1998, Liao *et al*. 2003, Ainsworth and Long 2005, Xu *et al*. 2006). Awareness of the regulatory effects of N on photosynthetic acclimation under $[CO_2]_e$ is not uniform. It is necessary to investigate the effect of N application on photosynthetic electron transport and distribution and this may help understanding the regulatory effect of N on photosynthetic acclimation at $[CO_2]_e$.

Photosynthesis is a coupled oxidation–reduction process (Lin *et al*. 2000), which includes photosynthetic carbon uptake and light energy utilization; N nutrition status has a significant effect on both photosynthetic carbon uptake and light energy assimilation (Pettersson and McDonald 1994, Reich *et al*. 2006). Zhang *et al*. (2006) reported that $[CO_2]_e$ reduced leaf N concentration of white birch seedlings; the high N availability increased photosynthetic linear electron transport to carboxylation and to oxygenation at both $[CO_2]_a$ and $[CO_2]_e$, but the effect on photosynthetic linear electron transport to carboxylation and oxygenation was greater under $[CO_2]_e$. However, the high N availability significantly decreased partitioning of photosynthetic linear electron transport to oxygenation, with more photosynthetic electrons distributed to photosynthetic assimilation. Similarly, Zhang *et al*. (2010) reported that although more excited light energy of wheat leaves would be dissipated as heat under $[CO_2]_e$, sufficient N increased the ratio of open PSII reaction centers and apparent rate of electron transport. This reduced photosynthetic linear electron transport to oxygenation, and thus more photosynthetic energy entered the photochemical process, resulting in a higher P_N. There have been some studies on the effect of N application on photosynthetic light energy transport and distribution at $[CO_2]_e$. Few studies have focused on the contribution of N to photosynthetic electron transport and distribution in Rubisco carboxylation and the oxygenation process, which is one of the most important parts of the electron transport chain in photosynthesis.

Current knowledge of photosynthetic acclimation is mostly based on the regulation of feedback inhibition of photosynthetic assimilation in chloroplasts (Bloom *et al*. 2002, Reich *et al*. 2006, Onoda *et al*. 2007). There has been only scanty research on the contributions of photosynthetic electron transport and partitioning on plant photosynthetic acclimation at $[CO_2]_e$. Therefore, further studies on the mechanisms of photosynthesis downregulation and its regulation by N would increase our understanding of photosynthetic acclimation under $[CO_2]_e$ from photosynthetic electron transport and partitioning aspects. Wheat is one of most important crops for human food industry, with a sowing area of around 0.22 billion ha. As a C_3 plant, wheat is sensitive to climate change. Thus, a study on photosynthetic acclimation of wheat under $[CO_2]_e$ might be useful for food security in future climate conditions. An open-top chamber system was used to simulate two atmospheric $[CO_2]$. Their effects on the electron transport rate and partitioning, triose phosphate utilization (TPU), and foliar N and Chl concentrations were investigated in plants grown under two N concentrations. This can help understanding effect of N application on photosynthetic acclimation.

Materials and methods

Plant material and culture conditions: The experiment was carried out at the experimental station of Gansu Academy of Agricultural Science, Lanzhou City, Gansu Province, China (36°06′07.13″N, 103°41′09.40″E and 1541 m above sea level). The average of total annual solar irradiance is 6.50×10^6 kJ cm^{-2}, average annual temperature is 11.2°C, and the relative air humidity in open-top chambers during the wheat growing season was 71.3%. Seeds were planted in white porcelain pots (35 cm high, top diameter of 30 cm, each filled with 8 kg of a growth medium). The growth medium was a jute soil with total N content of 1.1 g kg^{-1}, hydrolyzable N of 118.4 mg kg^{-1}, total phosphorus (P) of 0.7 g kg^{-1}, total K of 11.2 g kg^{-1}, soil organic matter of 1.3%, and field moisture capacity of 23.8%. In late March of both 2009 and 2010, wheat seeds were germinated in $[CO_2]_a$ and $[CO_2]_e$ (400 and 760 μmol mol^{-1}, respectively), the seedlings in the chamber with the same $[CO_2]$ were relatively uniform in size at the beginning of the experiment; the growth stages of wheat are shown in Fig. 1.

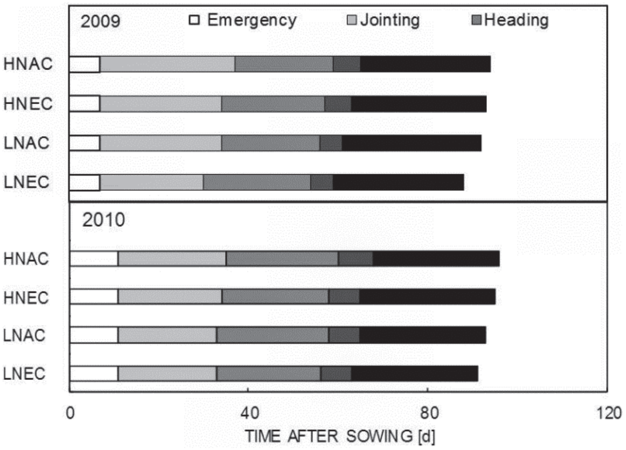

Fig. 1. Effects of atmospheric CO_2 concentration (400 and 760 μmol mol^{-1}; $[CO_2]_a$ and $[CO_2]_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on wheat development. LNEC – $[CO_2]_e$ + N0, HNEC – $[CO_2]_e$ + N200, LNAC – $[CO_2]_a$ + N0, and HNAC – $[CO_2]_a$ + N200.

Experimental design: The treatments consisted of $[CO_2]_a$ and $[CO_2]_e$ and two N application rates [0 (N0) and 200 (N200) mg N kg^{-1}(soil)]. The experiment was conducted in two environment-controlled, open-top chambers, one for each $[CO_2]$. The experiment was done in a randomized block design with each treatment consisting of 10 pots and 15 seedlings per pot. Fertilization was used before sowing. P fertilizer was applied at a rate of 100 mg kg^{-1}(soil). Day/night temperatures for all treatments were slightly different during both experimental years. Gravimetric water content of the soil was maintained around 18%. The seedlings were watered once every 2 d from March to early May and daily from mid-May to harvest. The experiment lasted from March 30 to June 29 in 2009 and from March 28 to July 5 in 2010. All measurements were taken at heading stage, the flag leaves were the 12th and 11th leaves at both N concentrations in both years. $[CO_2]_e$ had no significant effect on number of leaves, leaf area and plant height in both years.

Year	Day/night temperature [ºC]		Leaf area [cm^2]		Plant height [cm]	
	March–May	From June	N0	N200	N0	N200
2009	21–26/13–17	28–34/16–20	6.4–6.6	8.5–8.7	49.4–53.4	60.5–61.7
2010	18–25/9–16	26–33/15–21	6.3–6.4	8.3–8.4	51.6–53.3	60.0–62.1

Gas exchange and Chl fluorescence: Foliage photosynthetic gas exchange was measured with a *Li-Cor 6400* open gas-exchange system (*Li-Cor Environmental*, Lincoln, NE, USA). The environmental conditions in the leaf chambers were controlled automatically. Measurements of photosynthetic gas exchange were taken on the flag leaves from one plant per pot for three pots selected at random. P_N was measured sequentially at 0, 50, 100, 150, 200, 250, 300, 400, 600, 800, and 1,000 μmol(CO_2) mol^{-1} to establish P_N-C_i curves (where C_i is intercellular CO_2 concentration), $[CO_2]_a$ in the cuvette was controlled with a CO_2 mixer. Other environmental conditions for the measurements were 25°C air temperature; 1,000 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), and 50% relative humidity (RH). The automatic measurement system of *Li-Cor 6400* was selected to measure P_N-C_i curves: the designed maximum match time was 180 s, the minimum match time was 110 s at one single level of $[CO_2]$ and the data were recorded automatically after equilibration to a steady state (coefficient of variation < 2%). All measurements were made during 09:00–11:30 h. Nonlinear regression techniques, based on the equations of Sharkey (2007), were used to estimate a rate of respiration in the presence of light (R_L) for each P_N-C_i curve.

Chl fluorescence was measured with an *FMS-2* portable pulse-modulated fluorometer (*Hansatech Instruments*, Norfolk, UK). Data on the following variables were collected: maximal Chl fluorescence in the light (F_m'); the actual photochemical efficiency of PSII in the light (F_m' – F)/F_m' or ΔF/F_m'. After dark-acclimation for 30 min, opened examining light (< 0.05 μmol m^{-2} s^{-1}) was used to obtain F_o and then the foliage was immediately illuminated with a pulse of strong light (~6,000 μmol m^{-2} s^{-1}) for 800 ms to obtain F_m. After the measurement of F_v/F_m, the leaves were exposed to natural light for 30 min, then actinic light (1,000 μmol m^{-2} s^{-1}) was applied for 3 min to measure actual PSII efficiency under irradiance (Φ_{PSII}). The measurements were made between 09:00–11:30 h on the flag leaves of one plant per pot from five pots selected at random.

Leaf N and Chl: The flag leaves from five plants were sampled from the same plants used for the gas-exchange measurements. Total leaf N concentration was measured by the method of Nelson and Sommers (1973). N concentrations were expressed on a dry mass basis. Chl *a* and Chl *b* were extracted from flag leaf samples with N,N-dimethylformamide (DMF). Extraction was carried out in screw-capped glass bottles for 48 h at 4°C in darkness at an extraction ratio of 1:20 (w/v), and quantified by spectrophotometric analysis as described in Hartmut and Alan (1983).

Theoretical calculations: Based on Demmig-Adams *et al.* (1996), the simultaneously measured Chl fluorescence parameters are related as follows:

$$F_v'/F_m' = (F_m' - F_o')/F_m' \quad (1)$$

$$\Phi_{PSII} = (F_m' - F_s)/F_m' \quad (2)$$

$$q_P = (F_m' - F_s)/(F_m' - F_o') \quad (3)$$

$$NPQ = F_m/F_m' - 1 \quad (4)$$

where F_v'/F_m' is the maximal quantum yield of PSII under irradiance, q_P is photochemical fluorescence quenching, and NPQ is nonphotochemical fluorescence quenching.

Based on Haake *et al.* (1999) and Krall and Edward (1992), the simultaneously measured gas exchange and Chl fluorescence are related as follows:

$$J_F = (F_m' - F)/F_m' \times 0.5\ (PAR) \times 0.84 \quad (5)$$

$$J_F = 4\ V_c + 4\ V_o \quad (6)$$

$$P_N = V_c - 0.5\ V_o - R_D \quad (7)$$

where J_F is total electron transport rate of PSII; V_c and V_o are the rates of carboxylation and oxygenation of Rubisco, respectively, calculated according to Di Marco *et al.* (1994); and R_D is daytime dark respiration, calculated from the P_N-C_i curve.

The partitioning of total electron flow between carboxylation (J_c) and oxygenation (J_o) is determined by formulae (8) and (9) (Epron *et al.* 1995); V_c and V_o are calculated from formulae (10) and (11), which are deduced from formulae (6) and (7); TPU is determined from formula (12) (Sharkey *et al.* 2007):

$$J_c = 1/3[J_F + 8\ (P_N + R_D)] \quad (8)$$

$$J_o = 2/3[J_F - 4\ (P_N + R_D)] \quad (9)$$

$$V_c = 1/12[J_F + 9\ (P_N + R_D)] \quad (10)$$

$$V_o = 1/8[J_F - 4\ (P_N + R_D)] \quad (11)$$

$$TPU = (P_N + R_D)/3 \quad (12)$$

The leaf water-use efficiency (WUE) and photosynthetic N-use efficiency (PNUE) were determined as described by Zhang and Dang (2006):

$$WUE = P_N/E \quad (13)$$

$$PNUE = P_N/\text{foliar N concentration} \quad (14)$$

Statistical analysis: All analyses of variance (*ANOVA*) were performed in the software *SPSS* statistical package (*SPSS*, Chicago, USA); multiple comparisons were conducted by the least significant difference (LSD). Data were presented as means with standard errors.

Results

***In situ* gas exchange**: P_N and TPU increased as a result of greater N availability as well as $[CO_2]_e$ in both years (Table 1). The enhancement was in the range of 36.8–56.5%; all increases were significant compared with $[CO_2]_a$ or N0 application. However, g_s and E measurements showed an increase at the higher N availability, but a decrease at the $[CO_2]_e$, which was consistent in both years (Table 1). There was also significant interaction between CO_2 and N application for P_N, E, g_s, and TPU. For example, P_N value was more than 100% greater in the treatment of $[CO_2]_e$ and N application compared with $[CO_2]_a$ and N0 applications (Table 1).

Leaf Chl fluorescence parameters: N application significantly increased F_v'/F_m' at $[CO_2]_e$ (Fig. 2). At $[CO_2]_a$, the difference between high and low N availability was not significant. Compared to $[CO_2]_a$ level, $[CO_2]_e$ significantly decreased F_v'/F_m', with reductions of 20.1 and 16.7% in conditions of low N availability (N0) in 2009 and 2010, respectively. There was no significant difference in high N availability (N200) leaves. There was a significant interaction between $[CO_2]$ and N availability on F_v'/F_m'. The value of F_v'/F_m' was 23.6 and 19.1% higher in 2009 and 2010, respectively, under the $[CO_2]_a$ + N200 (HNAC) than in $[CO_2]_e$ + N0 (LNEC) treatment.

$[CO_2]_e$ decreased Φ_{PSII} by 21.8 and 24.0% in N0-treated leaves (Fig. 2) in 2009 and 2010, respectively, but it had no significant effect at N200 conditions. N application increased Φ_{PSII} under both $[CO_2]$, with N200 significantly increasing Φ_{PSII} by 36.0 and 40.0% under $[CO_2]_e$ in 2009 and 2010, respectively. q_P was also enhanced by N application under $[CO_2]_e$ by 15.4 and 17.1% in 2009 and 2010, respectively (Fig. 2). However, the effect of N on q_P was not significant under $[CO_2]_a$. $[CO_2]_e$ did not affect q_P regardless of N availability.

Unlike other Chl fluorescence parameters discussed above, NPQ showed a negative response to N application (Fig. 2), with values decreased by about 100% in both years under $[CO_2]_e$, as compared with N0 treatment. The reductions were less dramatic (14.9 and 29.7%) under $[CO_2]_a$. However, $[CO_2]_e$ significantly increased NPQ in N0 leaves, but decreased NPQ by 34.2 and 16.7% in N200 leaves in 2009 and 2010, respectively.

Photosynthetic energy transport: All five parameters of photosynthetic electron transport (total electron transport rate of PSII, J_F; electron transport partitioned to carboxylation, J_c; electron transport partitioned to photorespiration, J_o; Rubisco carboxylation rate, V_c; and Rubisco oxygenation rate, V_o) showed a positive and significant response to increased N availability, except in a few cases, where the response was either slightly negative or

Table 1. Effects of atmospheric CO_2 concentration (400 and 760 μmol mol^{-1}; $[CO_2]_a$ and $[CO_2]_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and triose phosphate utilization (TPU) in wheat leaves (mean ± SE, n = 3). Measurements were taken at the heading stage of wheat in 2009 and 2010. Values followed by *different letters* are significantly different at $p<0.05$ according to LSD test.

Parameters	Year	$[CO_2]_a$		$[CO_2]_e$	
		N0	N200	N0	N200
P_N [μmol(CO_2) m^{-2} s^{-1}]	2009	16.80 ± 2.19^d	23.18 ± 2.38^c	26.38 ± 1.39^b	36.88 ± 3.00^a
	2010	17.15 ± 2.34^c	24.00 ± 1.48^b	25.37 ± 2.55^b	35.67 ± 3.20^a
E [mmol(H_2O) m^{-2} s^{-1}]	2009	4.19 ± 0.34^c	7.63 ± 0.86^a	4.04 ± 0.38^c	6.05 ± 0.53^b
	2010	4.26 ± 0.41^c	6.97 ± 0.92^a	4.16 ± 0.26^c	6.10 ± 0.56^b
g_s [mol(H_2O) m^{-2} s^{-1}]	2009	0.26 ± 0.01^c	0.60 ± 0.05^a	0.23 ± 0.03^d	0.35 ± 0.03^b
	2010	0.26 ± 0.01^c	0.44 ± 0.05^a	0.21 ± 0.03^d	0.29 ± 0.04^b
TPU [μmol(CO_2) m^{-2} s^{-1}]	2009	7.51 ± 0.75^c	10.09 ± 0.89^b	10.59 ± 0.11^b	14.47 ± 1.32^a
	2010	7.82 ± 0.95^c	10.78 ± 1.41^b	10.13 ± 0.81^b	14.16 ± 0.12^a

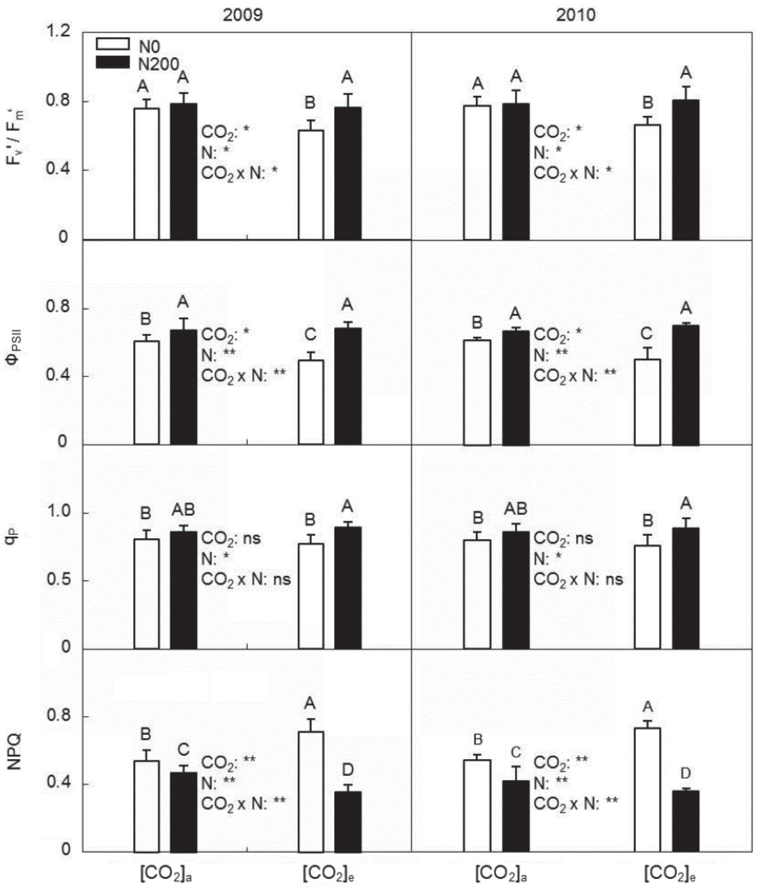

Fig. 2. Effects of atmospheric CO_2 concentration (400 and 760 μmol mol^{-1}; $[CO_2]_a$ and $[CO_2]_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on maximal quantum yield of PSII under irradiance (F_v'/F_m'), actual PSII efficiency under irradiance (Φ_{PSII}), photochemical fluorescence quenching coefficient (q_P), and nonphotochemical fluorescence quenching (NPQ) in wheat leaves (mean ± SE, *n* = 5). Measurements were taken at the heading stage of wheat in 2009 and 2010. Significance values (** – $p<0.01$; * – $p<0.05$, and ns – $p>0.05$) are based on *ANOVA*. Means sharing *the same letter(s)* do not differ significantly according to *Scheffe*'s *F*-test for *post-hoc* pairwise comparisons conducted when the interaction between $[CO_2]$ and N was significant for a given parameter.

Table 2. Effects of atmospheric CO_2 concentration (400 and 760 μmol mol^{-1}; $[CO_2]_a$ and $[CO_2]_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on total electron transport rate of PSII reaction center (J_F), electron transport rate of photochemistry (J_c), electron transport rate of photorespiration (J_o), Rubisco carboxylation rate (V_c), and Rubisco oxygenation rate (V_o) in wheat leaves (mean ± SE, *n* = 3). Measurements were taken at the heading stage of wheat in 2009 and 2010. Values followed by *different letters* are significantly different at $p<0.05$ according to LSD test.

Parameters	Year	$[CO_2]_a$		$[CO_2]_e$	
		N0	N200	N0	N200
J_F [μmol m^{-2} s^{-1}]	2009	255.8 ± 20.23^b	283.45 ± 22.4^a	208.9 ± 16.38^c	286.5 ± 19.03^a
	2010	259.0 ± 20.27^b	281.61 ± 23.9^a	210.6 ± 23.57^c	295.2 ± 27.52^a
J_c [μmol m^{-2} s^{-1}]	2009	145.3 ± 11.66^c	175.18 ± 13.5^b	154.3 ± 12.69^c	211.2 ± 14.03^a
	2010	148.9 ± 15.03^c	180.13 ± 14.5^b	151.3 ± 12.52^c	211.6 ± 13.51^a
J_o [μmol m^{-2} s^{-1}]	2009	110.4 ± 6.51^a	108.27 ± 7.2^a	54.6 ± 4.80^c	75.3 ± 7.39^b
	2010	110.1 ± 7.87^a	101.53 ± 7.8^a	59.4 ± 7.89^c	83.5 ± 5.18^b
V_c [μmol m^{-2} s^{-1}]	2009	32.9 ± 2.96^c	40.44 ± 3.3^b	36.9 ± 3.03^{bc}	50.5 ± 3.82^a
	2010	33.8 ± 4.01^c	41.88 ± 3.5^b	36.0 ± 3.17^c	50.3 ± 3.22^a
V_o [μmol m^{-2} s^{-1}]	2009	20.7 ± 1.72^a	20.26 ± 1.6^a	10.2 ± 0.89^c	14.1 ± 1.51^b
	2010	20.7 ± 1.73^a	19.02 ± 1.6^a	11.1 ± 1.83^c	15.7 ± 0.93^b

negligible (J_o and V_o at $[CO_2]_a$). Generally, the enhancement was greater under $[CO_2]_e$ compared with $[CO_2]_a$. For example, J_F increased by 37.2 and 40.2% under $[CO_2]_e$ but by 10.8 and 8.6% under $[CO_2]_a$ in 2009 and 2010, respectively (Table 2).

Compared with $[CO_2]_a$, J_F in N0 leaves decreased significantly, but slightly increased in N200 leaves at $[CO_2]_e$. At N200, J_c and V_c of leaves showed a positive and significant response to $[CO_2]_e$, but not at N0. However, $[CO_2]_e$ decreased J_o and V_o significantly in both years. For example, $[CO_2]_e$ increased J_c by 20.6 and 17.5% in N200 leaves but 6.2 and 1.6% in N0 leaves in 2009 and 2010, respectively (Table 2).

There was a significant interaction between $[CO_2]$ and N availability on J_c, J_o, V_c, and V_o. N200 together with $[CO_2]_e$ increased J_c and V_c but decreased J_o and V_o.

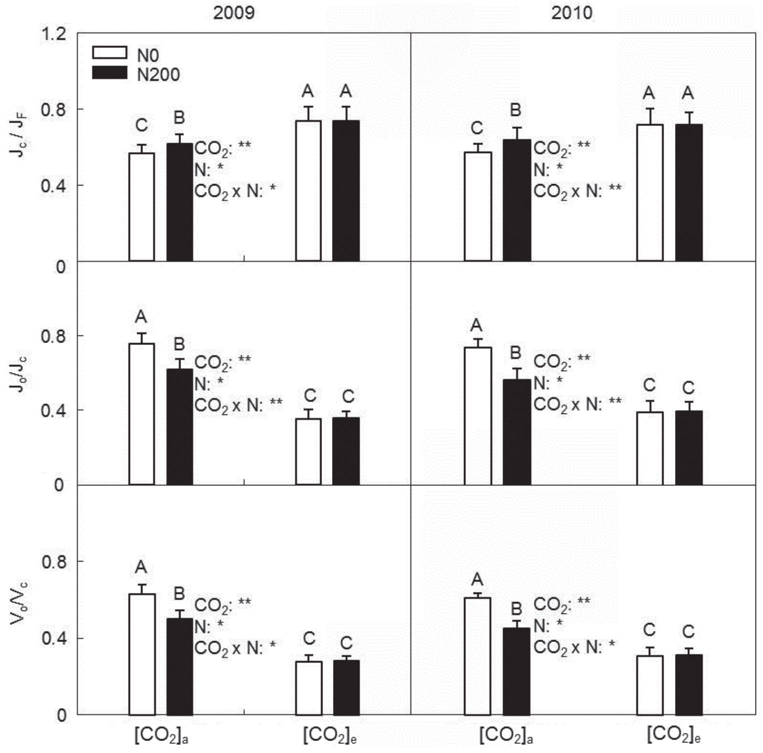

Fig. 3. Effects of atmospheric CO_2 concentration (400 and 760 μmol mol^{-1}; $[CO_2]_a$ and $[CO_2]_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on the ratio between photochemical electron transport rate and total electron transport rate of the PSII reaction center (J_c/J_F), the ratio between photorespiration electron transport rate and photochemical electron transport rate (J_o/J_c), and the ratio of Rubisco carboxylation/oxygenation rate (V_o/V_c) in wheat leaves (mean ± SE, $n = 3$). Measurements were taken at the heading stage of wheat in 2009 and 2010. Significance values (** – $p<0.01$; and * – $p<0.05$) are based on *ANOVA*. Means sharing *the same letter(s)* do not differ significantly according to *Scheffe*'s *F*-test for *post-hoc* pairwise comparisons conducted when the interaction between $[CO_2]$ and N was significant for a given parameter.

For example, the value of V_c in $[CO_2]_e$ + N200 (HNEC) treatment was 53.3 and 48.8% greater than in $[CO_2]_a$ + N0 (LNAC) treatment in 2009 and 2010, respectively. However, the value of J_o in LNEC treatment was the lowest among the treatment combinations.

Electron transport partitioning: At $[CO_2]_e$, N application did not affect the fraction of total electron transport partitioned to carboxylation (J_c/J_F), the ratio between electron transport partitioned to photorespiration *vs.* carboxylation (J_o/J_c), or the ratio of Rubisco carboxylation/oxygenation rates (V_o/V_c) (Fig. 3). However, under $[CO_2]_a$, N200 increased J_c/J_F, but decreased J_o/J_c and V_o/V_c. These trends were observed in both years. $[CO_2]_e$ increased J_c/J_F but decreased J_o/J_c and V_o/V_c regardless of N availability in both years. There was a significant interaction between $[CO_2]$ and N availability on J_c/J_F, J_o/J_c, and V_o/V_c. HNEC treatment increased J_c/J_F but decreased J_o/J_c and V_o/V_c. For example, the value of J_o/J_c and V_o/V_c in N0 leaves was the highest in the $[CO_2]_a$ treatment, but the lowest in the $[CO_2]_e$ treatment.

Leaf N content and PNUE: N application increased foliar N concentration and foliar Chl concentration regardless of CO_2 levels in both years (Fig. 4). $[CO_2]_e$ decreased leaf N concentration and foliar Chl concentration in leaves of both N treatments, but the decrease in foliar N concentration was larger than in foliar Chl concentration, indicating that more leaf N was distributed to Chl under $[CO_2]_e$. There was a significant interaction between $[CO_2]$ and N availability on leaf N concentration and foliar Chl concentration; the value was the highest for the HNAC treatment both in 2009 and 2010.

There was a general trend that PNUE and WUE decreased at higher N availability (Fig. 4). The reduction was greater at $[CO_2]_a$ than at $[CO_2]_e$. However, $[CO_2]_e$ increased PNUE and WUE regardless of N availability level – such increases were within the range of 51.5–100.7%. There was a significant effect on PNUE by the interaction between $[CO_2]$ and N availability; PNUE was 151.4 and 145.5% lower in HNAC treatment than in LNEC treatment in 2009 and 2010, respectively.

Discussion

***In situ* gas exchange**: HNEC enhances P_N synergistically in flag wheat leaves (Hunsaker *et al.* 2000, Zhang and Dang 2006). Stimulation of P_N in flag wheat leaves by N200 was greater under $[CO_2]_e$ than under $[CO_2]_a$ (Table 1), which agreed with many previously published results (Sage 1994, Zhang and Dang 2006, Yusuke *et al.* 2007, Zhang *et al.* 2010). However, $[CO_2]_e$ decreased *E* in N200 leaves, while showing no significant effect at N0. N200 significantly enhanced *E* under both $[CO_2]$, and the stimulation of *E* by N200 was greater at $[CO_2]_a$ than $[CO_2]_e$. This indicated that downregulation of *E* in response to $[CO_2]_e$ in wheat leaves depended on N availability; occurring in N200 but not in N0 leaves.

The downregulation of g_s in response to $[CO_2]_e$ was

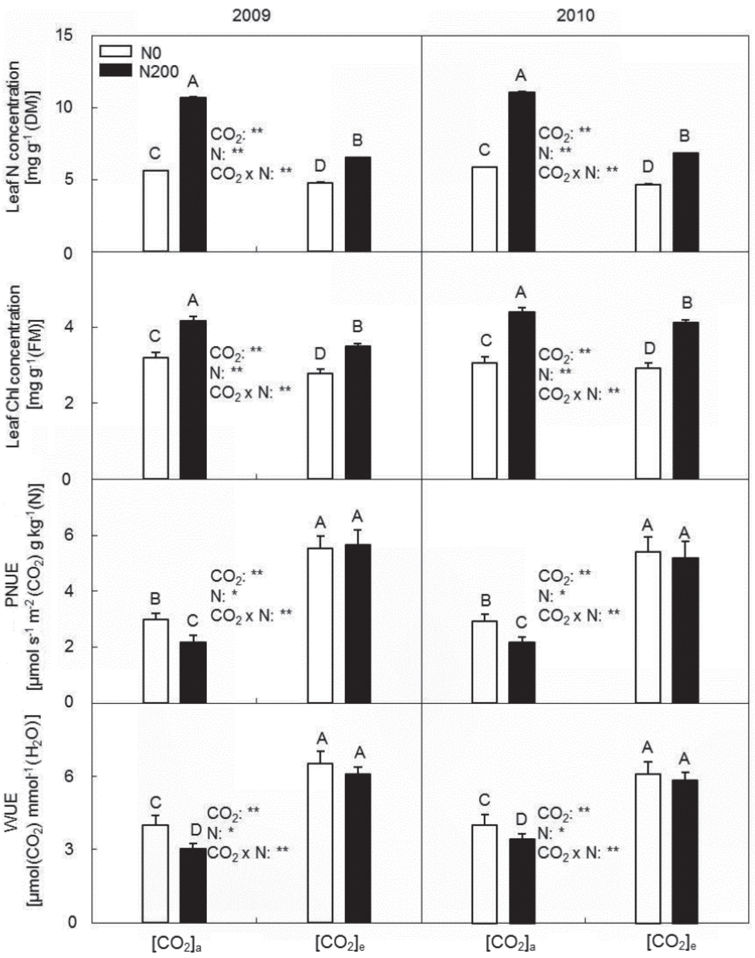

Fig. 4. Effects of atmospheric CO$_2$ concentration (400 and 760 μmol mol^{-1}; [CO$_2$]$_a$ and [CO$_2$]$_e$, respectively) and N application rates [0 and 200 mg(N) kg^{-1}(soil); N0 and N200, respectively] on leaf N concentration, leaf chlorophyll concentration, photosynthetic N-use efficiency (PNUE), and water-use efficiency (WUE) in wheat leaves (mean ± SE, n = 3). Measurements were taken at the heading stage of wheat in 2009 and 2010. Significance values (** – $p<0.01$; and * – $p<0.05$) are based on *ANOVA*. Means sharing *the same letter(s)* do not differ significantly according to *Scheffe*'s *F*-test for *post-hoc* pairwise comparisons conducted when the interaction between [CO$_2$] and N was significant for a given parameter.

found in both N availability treatments, decreasing by 8.9 and 22.5% in N0, and by 68.5 and 49.2% in N200 leaves in 2009 and 2010, respectively. TPU, an indicator of photosynthesis limitation by enzymes of the Calvin cycle, other than Rubisco (Sharkey *et al.* 2007), was significantly enhanced at N200 or [CO$_2$]$_e$ treatments. This indicated that N200 increased photosynthetic gas exchange and reduced the limitation of Calvin cycle enzymes on photosynthesis under [CO$_2$]$_e$. Consequently, N availability had a significant regulatory effect on photosynthetic gas exchange at [CO$_2$]$_e$. N200 reduced stomatal and nonstomatal limitations on gas exchange, resulted in increased P_N at [CO$_2$]$_e$ (Zhang and Dang 2006, Yusuke *et al.* 2007).

Leaf Chl fluorescence parameters: [CO$_2$]$_e$ decreased F_v'/F_m' in N0 wheat leaves, and N addition significantly increased F_v'/F_m' under [CO$_2$]$_e$ (Fig. 2). However, there was no significant difference in F_v'/F_m' between [CO$_2$]$_e$ and [CO$_2$]$_a$ treatments in N200 leaves. Φ_{PSII} can indicate a large portion of inactive reaction centers due to oxidation of D_1 proteins (Marwood *et al.* 2001, Baker 2008). [CO$_2$]$_e$ significantly decreased Φ_{PSII} in N0 leaves, but had no effect in N200 flag leaves; sufficient N increased Φ_{PSII} under both [CO$_2$] treatments (Calatayud *et al.* 2006). The stimulation of Φ_{PSII} was greater at [CO$_2$]$_e$ than [CO$_2$]$_a$. This indicated that N200 could increase the portion of active PSII reaction centers (Baker and Rosenqvist 2004), but N deficiency induced downregulation of photosynthetic energy utilization of PSII in response to [CO$_2$]$_e$ (Ainsworth and Long 2005).

q_P, which is related to the portion of PSII centers that are open (Pastenes and Horton 1996, Baker 2008), was not significantly affected by [CO$_2$], but increased in HNEC leaves. However, N200 decreased and N0 increased NPQ in wheat flag leaves under [CO$_2$]$_e$. N availability is a critical factor relating to the photosynthetic energy utilization functions of PSII centers (Zhang and Dang 2006). N200 elevated the ratio of opened PSII centers, reduced heat loss of excited energy by antennae, and enhanced more photosynthetic electron transport through PSII centers to downstream under [CO$_2$]$_e$.

Photosynthetic energy transport: The total electron transport rate of PSII reaction centers (J_F), determined by Φ_{PSII} (Krall and Edward 1992, Haake *et al.* 1999), changed in a fashion similar to Φ_{PSII} under different N availabilities and [CO$_2$] treatments. Total electron transport rate decreased under [CO$_2$]$_e$ with N0 flag wheat leaves, but it did not change at N200 (Table 2).

Consequently, the total electron transport rate of the PSII reaction centers was downregulated in response to $[CO_2]_e$ in the N0 treatment, but this acclimation was absent under N200. This suggests that N availability was a key factor determining the acclimation of J_F; N200 supported opening PSII reaction centers, with more electrons transported through PSII reaction centers downstream under $[CO_2]_e$ (Zhang and Dang 2006).

$[CO_2]_e$ improved electron transport partitioned to carboxylation (J_c) but decreased the partition to photorespiration (J_o) in N200 treatment, which agreed with results of Ainsworth and Long (2005). However, in N0 leaves, J_c did not change and J_o decreased significantly. N200 increased both J_c and J_o under $[CO_2]_e$; however, J_c increased and J_o did not change significantly under $[CO_2]_a$ (Table 2). This indicated that the carboxylation electron transport rate was upregulated and the photorespiration electron transport rate was downregulated in response to $[CO_2]_e$ in the N200 treatment. However, in N0 leaves, this acclimation of J_c was absent, and J_o was downregulated.

Similar to J_c and J_o, $[CO_2]_e$ significantly increased V_c and decreased V_o in the N200 treatment. However, in N0 leaves, V_c did not change, while V_o decreased significantly. N200 increased both V_c and V_o under $[CO_2]_e$; however, under $[CO_2]_a$, V_c increased and V_o did not change (Table 2). This suggested that the carboxylation rates of Rubisco were upregulated, whereas the oxygenation rate of Rubisco was downregulated in response to $[CO_2]_e$ in the N200 treatment. In N0 leaves, this acclimation of V_c was absent, while V_o was downregulated. Despite N200 increasing both carboxylation and oxygenation energy dissipation, $[CO_2]_e$ caused more RuBP to be carboxylated at the expense of oxygenation (Sharkey *et al.* 2007), resulting in a high photosynthetic rate under N200 and $[CO_2]_e$.

Electron transport partitioning: $[CO_2]_e$ had a significant effect on photosynthetic electron transport partitioning in wheat leaves, the ratio maximum Rubisco carboxylation rate/maximum electron transport rate decreased under $[CO_2]_e$ and N0 (Ainsworth and Long 2005). Zhang and Dang (2006) reported that $[CO_2]_e$ reduced J_o/J_F, but N availability had no significant effect on J_o/J_F. In the present study, N availability and $[CO_2]$ affected electron partitioning (Fig. 3); $[CO_2]_e$ stimulated the proportion of the total electron flow partitioned to photochemical processes (J_c/J_F), but suppressed partitioning to photorespiration (J_o/J_c) and Rubisco oxygenation (V_o/V_c). N200 stimulated J_c/J_F, and suppressed J_o/J_c and V_o/V_c under $[CO_2]_a$, but N availability had no significant effect on electron partitioning under $[CO_2]_e$. This indicated that as the limitation of photosynthetic electron partition, which was caused by RuBP regeneration, was lowered, the more photosynthetic electrons were allocated to photochemical and carboxylation reactions under $[CO_2]_e$. This conclusion is consistent with the findings of Zhang and Dang (2006), and supported by those of Sharkey *et al.* (2007). In addition, N200 did not increase the partition ratio of photorespiration and carboxylation reactions, but increased the total electron transport capacity relative to carboxylation capacity under $[CO_2]_e$. More photosynthetic electron transport to the carboxylation process resulted in a higher photosynthetic rate under N200 and $[CO_2]_e$.

Leaf N content and PNUE: Our results supported the theory that $[CO_2]_e$ can lead to a rapid depletion of soil N by pot-grown plants (Pettersson and Mc Donald 1994). In our experiment, $[CO_2]_e$ significantly reduced foliar N concentration even at the high N treatment. Although N200 raised foliar N concentration, such an increase was inadequate to replenish the foliar N concentration compared with $[CO_2]_a$ treatment (Fig. 4). Foliar Chl concentration also declined under the $[CO_2]_e$ treatment, but the decrease was not as severe as that for foliar N concentration. This suggests that foliar N allocation changed under $[CO_2]_e$. More N was distributed to photosynthetic apparatus (Yusuke *et al.* 2007), this is the biochemical basis for the decrease in foliar N concentration not resulting in photosynthetic downregulation (Long *et al.* 2004, Ainsworth and Rogers 2007, Huang *et al.* 2007). It is also the reason why total electron transport rate of the PSII reaction center was still high in N200 wheat leaves, in which N concentration decreased significantly under $[CO_2]_e$ (Ellsworth *et al.* 2004).

PNUE raised significantly under $[CO_2]_e$; N application increased PNUE under $[CO_2]_e$, but decreased PNUE under $[CO_2]_a$. This result also indicated that more N was distributed to Chl, and thus the decreased foliar N concentration did not cause photosynthetic downregulation under $[CO_2]_e$. $[CO_2]_e$ has been shown to increase assimilation and decrease g_s and E (Wullschleger *et al.* 2002, Katul *et al.* 2010) and increase leaf WUE. In the present study, based on increased P_N and decreased E, WUE in N200 leaves increased at $[CO_2]_e$ compared with $[CO_2]_a$ by 100.7 and 70.1% in 2009 and 2010, respectively (Fig. 4). The interaction between $[CO_2]$ and N availability had a significant effect on WUE at the heading stage of wheat: WUE in LNEC compared with HNAC treatment was 115.1 and 77.3% higher in 2009 and 2010, respectively.

Conclusion: Chl concentrations declined less than foliar N concentrations under $[CO_2]_e$, thus, the total electron transport rate of PSII reaction centers did not decrease in N200 wheat leaves. Photorespiration rate and Rubisco oxygenation rates were inhibited under $[CO_2]_e$, stimulating the proportion of the total electron flow partitioned to photochemical processes and carboxylation, thus more photosynthetic electrons were transported to the carboxylation process. N application increased both photorespiration and oxygenation rates of Rubisco, but had no significant effect on photosynthetic electron partitioning and it reduced the limitation of Calvin cycle enzymes on photosynthesis. This suggests that N availability and $[CO_2]_e$ had significant interactive effects on photosynthetic electron transport rate and partitioning; high N availability could promote more photosynthetic energy to the carboxylation process, resulting in higher photosynthetic rates under high N availability and $[CO_2]_e$.

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