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Higher Atmospheric CO_2 Levels Favor C_3 Plants Over C_4 Plants in Utilizing Ammonium as a Nitrogen Source

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Photosynthesis of wheat and maize declined when grown with NH_4^+ as a nitrogen (N) source at ambient CO_2 concentration compared to those grown with a mixture of NO_3^- and NH_4^+, or NO_3^- as the sole N source. Interestingly, these N nutritional physiological responses changed when the atmospheric CO_2 concentration increases. We studied the photosynthetic responses of wheat and maize growing with various N forms at three levels of growth CO_2 levels. Hydroponic experiments were carried out using a C_3 plant (wheat, *Triticum aestivum* L. cv. Chuanmai 58) and a C_4 plant (maize, *Zea mays* L. cv. Zhongdan 808) given three types of N nutrition: sole NO_3^- (NN), sole NH_4^+ (AN) and a mixture of both NO_3^- and NH_4^+ (Mix-N). The test plants were grown using custom-built chambers where a continuous and desired atmospheric CO_2 (C_a) concentration could be maintained: 280 μmol mol^{-1} (representing the pre-Industrial Revolution CO_2 concentration of the 18th century), 400 μmol mol^{-1} (present level) and 550 μmol mol^{-1} (representing the anticipated futuristic concentration in 2050). Under AN, the decrease in net photosynthetic rate (P_n) was attributed to a reduction in the maximum RuBP-regeneration rate, which then caused reductions in the maximum Rubisco-carboxylation rates for both species. Decreases in electron transport rate, reduction of electron flux to the photosynthetic carbon [*Je(PCR)*] and electron flux for photorespiratory carbon oxidation [*Je(PCO)*] were also observed under AN for both species. However, the intercellular (C_i) and chloroplast (C_c) CO_2 concentration increased with increasing atmospheric CO_2 in C_3 wheat but not in C_4 maize, leading to a higher *Je(PCR)/ Je(PCO)* ratio. Interestingly, the reduction of P_n under AN was relieved in wheat through higher CO_2 levels, but that was not the case in maize. In conclusion, elevating atmospheric CO_2 concentration increased C_i and C_c in wheat, but not in maize, with enhanced electron fluxes towards photosynthesis, rather than photorespiration, thereby relieving the inhibition of photosynthesis under AN. Our results contributed to a better understanding of NH_4^+ involvement in N nutrition of crops growing under different levels of CO_2.

Keywords: atmospheric CO_2, ecophysiology, electron transport, NH_4^+ stress, photosynthesis, *Triticum aestivum*, Zea mays

INTRODUCTION

The application of chemical nitrogen (N) fertilizers has greatly increased global crop yields and decreased world hunger over the past five decades (Gong et al., 2011). However, only 30–40 % of applied N is utilized by crops; most is lost in numerous ways, including run-off, leaching, denitrification and volatilization, which together lead to a range of environmental problems (Richter and Roelcke, 2000; Xing and Zhu, 2000). Thus, increasing plant nitrogen use efficiency (NUE) is crucial for the development of sustainable agriculture. Unlike nitrate (NO_3^-), ammonium (NH_4^+) can be assimilated by plants without further chemical reduction (Mehrer and Mohr, 1989; Onoda et al., 2004). NH_4^+ can be provided by both manure and urea fertilizers (Xu et al., 2012; Coskun et al., 2017). A promising future strategy for improving agronomic NUE is the application of stabilized-NH_4^+-based fertilizers together with other active compounds, such as nitrification inhibitors, which can inhibit the nitrification of NH_4^+, thereby maintaining a high soil N content in the form of NH_4^+ over extended periods (IPCC, 2007; Ariz et al., 2011). Some crops, including wheat and maize, are able to grow well when provided with a mixture of NO_3^- and NH_4^+ (Mix-N), or NO_3^- as the sole N source (NN) (Miller and Cramer, 2005). Under certain environmental conditions, NH_4^+ may reduce growth by decreasing photosynthesis, thereby lowering crop productivity (Britto and Kronzucker, 2002). Since urea and NH_4^+-based N fertilizers are used commonly to support the growth of cereals, vegetables and fruits, a better understanding of the toxic effects of NH_4^+ in plant nutrition should facilitate better crop productivity (Miller and Cramer, 2005; Fernández-Crespo et al., 2012).

Photosynthesis is a synergistic process that involves electron harvesting, transport, and utilization (Kühlbrandt et al., 1994). Photosynthetic electron transport typically involves two reaction centers: photosystems I and II (PSI and PSII, respectively). The D1 protein of PSII is sensitive to NH_4^+, and a loss of PSII function occurs when NH_4^+-based fertilizers are applied in excessive quantities (Drath et al., 2008). When this occurs, impairment of the photosynthetic electron transport chain will lead to a decrease in photochemical efficiency (Φ_{PSII}) and the electron transport rate (J_t), in turn leading to a deficiency in NADPH and ATP for CO_2 assimilation (Wang et al., 2019). The atmospheric CO_2 concentration (C_a) has increased from 280 μmol mol^{-1} in pre-industrial times to 400 μmol mol^{-1} at present, and is expected to reach 550 μmol mol^{-1} by the 2050 s (IPCC, 2013). The elevation of CO_2 concentration at the sites of Rubisco carboxylation alters plant photosynthetic sensitivity, potentially modulating sensitivity to a diversity of N sources. Due to the importance of food production security and crops in the global carbon cycle, an improved understanding of C_a changes on the N nutrition of C_3 and C_4 plants will become more and more crucial (Lloyd and Farquhar, 1996; Ghannoum et al., 2000). In general, plants with the C_4 photosynthetic pathway have anatomical and biochemical traits that increase CO_2 levels around the carboxylating Rubisco enzyme (Hatch and Slack, 1966). Bloom et al. (2014) and Dier et al. (2018) showed that NO_3^- assimilation is inhibited by elevated CO_2 concentrations in field-grown C_3 wheat plants. We postulated that an elevated C_a increases photosynthesis to produce more carbon skeletons, which in turn would increase NH_4^+ assimilation, thereby ameliorating the possible physiological stress of having excess free NH_4^+ in C_3 plants. The detailed comparative physiological responses of C_3 and C_4 plants to NH_4^+ fertilization under elevated C_a are still not fully understood (Bloom et al., 2002, 2010; Cousins and Bloom, 2003; Bloom, 2015).

A wide variety of equipment, including open-top chambers (OTC), controlled-environment (CE) systems, and free-air CO_2 enrichment (FACE) systems, has been used to study the effects of elevated C_a. In the OTC system, plants are held in a chamber with an open top that facilitates gas exchange with the atmosphere (Owensby et al., 1999). However, the temperature is generally higher inside the chamber than outside, inevitably increasing plant transpiration, which influences plant growth rates. The FACE system minimally perturbs the plant growth environment and is suitable for long-term experiments under elevated C_a. However, the FACE system could not be used to study the effects of sub-ambient CO_2 concentrations. Controlled environment experiments can also be performed in greenhouses and artificial growth chambers (Yong et al., 2000: Aranjuelo et al., 2009; Kanemoto et al., 2009; Robredo et al., 2011). To design a system having minimal impact on natural temperature, light and seasonality, while also having the capacity to provide either sub-ambient or elevated levels of CO_2, we custom-fabricated experimental chambers in which the inside temperature and humidity were kept similar to outside open field conditions while regulating the C_a.

Our primary objective was to improve understanding of (i) the physiological mechanisms underlying the photosynthetic inhibition caused by NH_4^+ nutrition and (ii) the mechanism by which C_a affects the NH_4^+ tolerance of C_3 and C_4 plants. We therefore studied the photosynthetic responses of a C_3 plant (wheat, *Triticum aestivum* L.) and a C_4 plant (maize, *Zea mays* L.). Both these species prefer NO_3^- as the N nutrient source and we grew them under three C_a concentrations (280, 400, or 550 μmol mol^{-1}) combined with three forms of N

Abbreviations: Γ*, the CO2 compensation point related to Ci; φ, apparent quantum yield; ΦPSII, quantum efficiency of PSII; Ca, atmospheric CO2 concentration; Cc, chloroplastic CO2 concentration; CE, carboxylation efficiency; Ci, intercellular CO2 concentration; Cm, CO2 concentrations in the mesophyll cells; Cs, CO2 concentrations in the bundle sheath; C treatment, the CO2 concentration treatment of each sample; FACE, free-air CO2 enrichment; Fm, the maximum chlorophyll fluorescence with dark-adaptation; Fm', the maximum fluorescence with light-adaptation; Fo, the minimum chlorophyll fluorescence with dark-adaptation; Fo', the minimum chlorophyll fluorescence with light-adaptation; Fs, steady state fluorescence with light-adaptation; Fv/Fm, maximum quantum efficiency of PSII; gm, mesophyll conductance; gs, stomatal conductance; Ja, alternative electron flux; Jcmax, maximum carboxylation rates limited by RuBP regeneration; Je(PCO), electron fluxes to photosynthetic carbon oxidation; Je(PCR), electron fluxes to photorespiratory carbon reduction; Jmax, maximum electron transport rates; LHC, light-harvesting complex; Mix-N, a mixture of both NO3– and NH4+ source; NPQ, non-photochemical quenching; NUE, nitrogen use efficiency; OTC, open-top chambers; Pn, net photosynthetic rate; PPFD, photon flux intensity; PSII, photosystem II; PVC, polyvinyl chloride; qL, photochemical quenching; Rd, mitochondrial respiration rate in the light; ROS, reactive oxygen species; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; vc, the carboxylation rate; Vcmax, maximum carboxylation rate limited by Rubisco; vo, the oxygenation rate; Vpdl, the vapor pressure deficit; Vpmax, the maximal rate of PEP carboxylation; wc, the potential Rubisco carboxylation rate; wj, the potential RuBP regeneration rate; wp, the potential triose-phosphate utilization rate.

nutrition: Mix-N, NN and sole NH_4^+ nitrogen (AN). Our other objective was to provide new knowledge facilitating (i) directed breeding programs aiming to produce N-efficient cultivars, and (ii) the development of sustainable crop N management strategy to adapt to a future with elevated C_a, as predicted by current models of global climate change.

MATERIALS AND METHODS

Plant Materials and Experimental Design

Wheat (*T. aestivum* cv. Chuanmai 58) and maize (*Z. mays* cv. Zhongdan 808), two common crop species in Chongqing, China, were grown under hydroponic experimental conditions. Seeds of both species, of uniform size, were sterilized in 20% (v/v) H_2O_2 for 10 min, rinsed with distilled water, and germinated in darkness in culture dishes covered with wet sterile gauze. When the cotyledons were 1.0 cm long, the seedlings were transferred to silica sand (previously soaked in 1% HCl for 2 days, followed by flushing with copious amounts of water to remove all traces of HCl) and watered twice daily with distilled water. Uniform 14-day-old (two-leaf stage) seedlings were transplanted into opaque plastic growth containers containing a modified Hoagland's solution (Wang et al., 2016a; Gao et al., 2018), with three N sources: Mix-N, NN or AN. The composition of the Mix-N solution was as follows: macronutrients were provided as 5.0 mM N in the form of $Ca(NO_3)_2$, KNO_3 and $(NH_4)_2SO_4$ (the ratio of NO_3^- to NH_4^+ in the Mix-N was 3 : 2), 3.0 mM K in the form of KH_2PO_4 and KNO_3, 1.5 mM Ca as $Ca(NO_3)_2$ and $CaCl_2$, 1.0 mM Mg as $MgSO_4$, 1.0 mM P as KH_2PO_4, and 0.6 mM Na as NaCl. Micronutrients were provided as 0.1 mM Fe as Fe-EDTA, 455×10^{-3} mM Mn as $MnSO_4$, 38.1×10^{-6} mM Zn as $ZnSO_4$, 15.6×10^{-6} mM Cu as $CuSO_4$, 2.31×10^{-3} mM B as H_3BO_3, and 6.2×10^{-6} mM Mo as MoO_3. Macronutrients were provided in the NO_3^--source solution as 5.0 mM N in the form of $Ca(NO_3)_2$ and KNO_3, 3.0 mM K in the form of KH_2PO_4 and KNO_3, 1.5 mM Ca as $Ca(NO_3)_2$, 1.0 mM Mg as $MgSO_4$, 1.0 mM P as KH_2PO_4, and 0.5 mM Na as NaCl. Macronutrients were provided in the NH_4^+-source solution as 5.0 mM N in the form of $(NH_4)_2SO_4$, 3.0 mM K as KH_2PO_4 and K_2SO_4, 1.5 mM Ca as $CaCl_2$ and $CaSO_4$, 1.0 mM Mg as $MgSO_4$, 1.0 mM P as KH_2PO_4, and 0.5 mM Na as NaCl. Micronutrients in NN or AN source solution were identical to those in the Mix-N source solution; and then kept in chambers (**Figure 1A**) with the following CO_2 levels: 280 μmol mol^{-1} [pre-industrial revolution (i.e., 1840) concentration], 400 μmol mol^{-1} (current level), and 550 μmol mol^{-1} (projected concentration by the 2050 s) (IPCC, 2013). The temperature and humidity inside the chambers were automatically maintained to match those of the atmosphere outside (**Figure 1B**). During the night, the CO_2 gradients were held at concentrations 150 ± 1 μmol mol^{-1} above daytime levels (Anderson et al., 2001).

A nitrification inhibitor (dicyandiamide, 1 mM) was added to the nutrition solutions to prevent microbial oxidation of NH_4^+. The pH of the Hoagland's solution was adjusted daily to 5.5 using 0.1 mM NaOH for the plants treated with NH_4^+, or with 0.1 mM H_2SO_4 for those treated with NO_3^-. The N concentrations were kept constant by replacing the media at 3-day intervals; aeration was provided continuously. We used three CO_2 treatments, with three replicates each in separate chambers (9 chambers in total), and deployed three N treatments for each species in each chamber. Ideally, each experiment should be repeated at least once in every chamber, for each of the planned treatments, to eliminate any intrinsic technical effect of each chamber on plant growth; this represented a potential small shortcoming of the current study in our facility.

Experimental Field Chambers

The automatically CE facility consisted of a CO_2 control system (2543CN; Shengsen Corp., Qingdao, China; **Supplementary Figures S1-1**) and a CO_2 generator (12864; Shengsen Corp.; **Supplementary Figure S1-2**). The CO_2 generator comprised of several components, i.e., an electric connection point pressure meter (STC90C516RD; Shengsen Corp.), pressure sensors (13864; Shengsen Corp.), and Na_2CO_3 and H_2SO_4 feeding inlets (**Supplementary Figures S1-5, 6**), where the CO_2 was generated according to the following equation: $Na_2CO_3+H_2SO_4= Na_2SO_4+H_2O + CO_2$ The CO_2 generator was connected to the CO_2 control system to maintain the CO_2 concentration in the chambers within a desired set range; CO_2 was delivered through pipes into each chamber.

The chambers were built using toughened glass (10 mm thick, 99% transmittance) walls and roofs (**Supplementary Figure S1-13**). The floors were covered using a polyvinyl chloride (PVC) material (**Supplementary Figure S1-7**). Each chamber measured 1,500 × 1,000 × 2,000 mm (length × width × height). CO_2, temperature and humidity sensors were mounted on both the outer and inner surfaces of the walls (**Supplementary Figures S1-8–10**). The environmental control mechanism in this system operated automatically and regulated the internal CO_2 concentration (±2.5 μmol mol^{-1}), air temperature (±0.5°C) and humidity (±5%) (**Figure 1B**). The CO_2 control system sensed and assessed the chamber environmental data; these data were later used to regulate the CO_2 injection process and attaining the desired chamber CO_2 levels. When the CO_2 concentration in a chamber exceeded the set concentration, the air was filtered through 1.0 mol mol^{-1} NaOH solution using a pump controlled by a mini-computer. When the humidity of a chamber exceeded the set concentration, the air was filtered through dry calcium carbonate using a separate pump controlled by the mini-computer.

Plant Sampling

The seedlings were sampled between 10:00 and 11:00 at the 21st day of the experiment. Plant organs were separated into two portions: the first was immersed in liquid-N and then stored at –80°C for later chemical analyses, and the second portion was oven-dried at 105°C for 20 min, and then at 75°C for at least 48 hours. The dried material was used later for different chemical analyses. Fresh leaf area was measured using a leaf area scanning device (Li-3000; Li-Cor Inc., Lincoln, NE, United States).

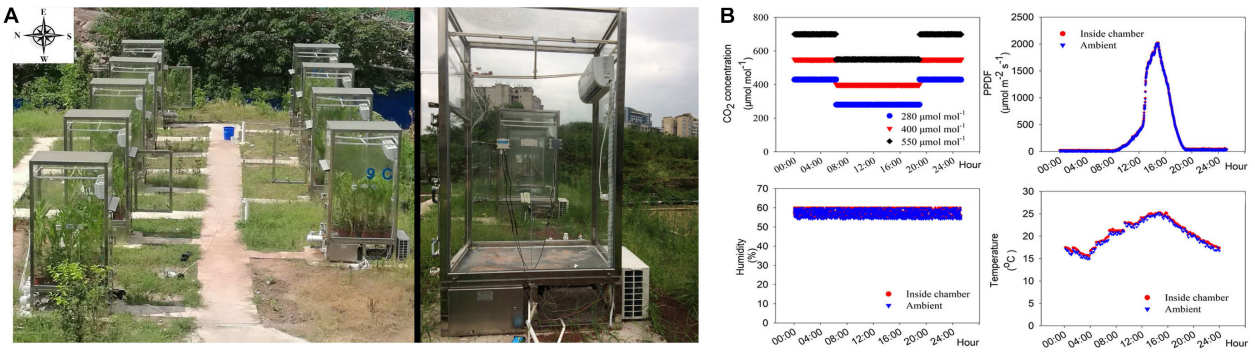

FIGURE 1 | Custom-built chambers **(A)** that automatically maintained desired CO_2 levels, while temperature and humidity of the chambers were similar to the outside atmospheric environment and **(B)** mean CO_2 concentrations maintained inside the CO_2 chamber; intensity of photon flux intensity (PPFD); humidity and temperature inside and outside the chambers over a period of 21 days of growth.

Gas-exchange Measurements

After 21 days of growth under different N source and CO_2 level conditions, we measured gas exchange and chlorophyll fluorescence simultaneously on the first fully developed leaves during the morning (09:00–11:00) using a Li-Cor 6400 infrared gas analyzer (Li-Cor 6400; Li-Cor Inc.). The leaf temperature during measurements was maintained at 25.0 ± 0.5^{o}C. Leaves were illuminated with a steady red and blue light source at a photosynthetic photon flux density (PPFD) of 1,500 μmol m^{-2} s^{-1} (Yong et al., 2000, 2010). The reference CO_2 concentrations in the cuvettes matched the treatment CO_2 concentrations to which samples had been previously subjected ($C_{treatment}$), i.e., 280 ± 2.5, 400 ± 2.5 or 550 ± 2.5 μmol mol^{-1}. The vapor pressure deficit (Vpdl) was 1.1 ± 0.05 kPa, and the relative humidity was in the range 55–65%. The gas exchange instrument was calibrated each day before the measurements and matched at least twice a day (between the curves). Data were recorded after sample acclimation in the cuvette for at least 15 min.

Two types of curves were plotted: net photosynthesis (A_n) *vs.* intercellular CO_2 concentrations (C_i, **Supplementary Figure S2**), and A_n *vs.* PPFD. Simultaneous measurements of chlorophyll fluorescence and parameters for plotting the A/C_i curves were made on the same leaf using the Li-Cor 6400 infrared gas analyzer. Leaf temperature, PPFD, Vpdl and relative humidity were maintained as indicated above. Prior to measurement, leaves were held in the cuvette at a reference CO_2 concentration of $C_{treatment}$ for at least 10 min. The reference CO_2 concentration was controlled across a series of $C_{treatment}$ values: 200, 150, 100, 50, 400, 600, 800, 1,000, 1,200, and 1,500 μmol mol^{-1}. Data were collected after the prevailing CO_2 had reached a steady state (2–3 min).

Method for Determining Photosynthetic Parameters of C_3 and C_4 Plants

The parameters for the C_3 wheat plant photosynthesis model were calculated using the equations of Farquhar et al. (1980); Long and Bernacchi (2003), Gao et al. (2018), and Wang et al. (2019).

According to the photosynthesis model that we used for C_4 maize plants (von Caemmerer and Farquhar, 1999), the rates of phosphoenolpyruvate (PEP) and Rubisco carboxylation (V_p and V_c, respectively) are the major determinants of the net CO_2 assimilation rate. The Rubisco carboxylation rate (V_{cmax}), the maximal rate of PEP carboxylation (V_{pmax}), maximum RuBP-regeneration rate (J_{max}) and CO_2 concentration in the bundle sheath (C_s) for maize were calculated using the following equations. The photosynthetic rate was expressed mathematically as:

$$A = V_p - R_m - L \tag{1}$$

and

$$A = V_c - 0.5V_0 - R_d \tag{2}$$

where R_m is the mitochondrial respiration of the mesophyll cells, L is the rate of CO_2 leakage from the bundle sheath into the mesophyll, and R_d is the mitochondrial respiration rate in the light.

$$L = g_{bs} \times (C_s - C_m) \tag{3}$$

$$C_m = C_i - \frac{A}{g_i} \tag{4}$$

where g_{bs} is the bundle sheath conductance for CO_2, g_i is the mesophyll conductance for CO_2, C_s is the CO_2 concentration in the bundle sheath, and C_m is the CO_2 concentration in the mesophyll cells. V_o is the rate of Rubisco oxygenation:

$$V_0 = \frac{2\gamma * O}{C_s} \times V_c \tag{5}$$

where γ^* is one half of the reciprocal of Rubisco specificity ($S_{c/o}$), and O is the oxygen concentration in the bundle sheath cells, which matches the oxygen concentration in the mesophyll cells. By fitting equation (3) to equation (1), and equation (5) to equation (2), we obtained the following expressions:

$$A = V_p - R_m - g_{bs} \times (C_s - C_m) \tag{6}$$

and

$$A = V_c \times \left(1 - \frac{\gamma * O}{C_s}\right) - R_d \quad (7)$$

V_p and V_c depend on V_{cmax}, V_{pmax}, J_{max}, the Michaelis constants for O_2 and CO_2 (K_o, K_c and K_p), and the relative specificity of Rubisco ($S_{c/o}$).

To calculate V_{cmax} and V_{pmax}, we used the enzyme-limited expressions of V_p and V_c:

$$V_p = \frac{C_m V_{p\max}}{C_m + K_p} \quad (8)$$

$$V_c = \frac{C_s V_{c\max}}{C_s + K_p\left(1 + \frac{0}{K_0}\right)} \quad (9)$$

By fitting equation (8) to equation (6), and equation (9) to equation (7), we obtained the following expressions:

$$A = \frac{C_m V_{p\max}}{C_m + K_p} - R_m - g_{bs} \times (C_s - C_m) \quad (10)$$

and

$$A = \frac{C_s V_{c\max}}{C_s + K_c\left(1 + \frac{0}{K_0}\right)} \times \left(1 - \frac{\gamma * O}{C_s}\right) - R_d \quad (11)$$

In equations (4), (10) and (11), g_i, g_{bs}, R_m, K_p, K_c, K_o, O, R_d and γ^* were constant parameters at a given temperature [as described by von Caemmerer and Farquhar (1999)], A and C_i were measured values, and C_m, C_s, V_{cmax} and V_{pmax} were unknowns. Two pairs of (A, C_i) (with C_i limited to 40–80 μmol mol^{-1}) were then inserted into two sets of equations (5), (8) and (9), following which we obtained six equations and six unknowns (V_{cmax}, V_{pmax}, C_s1, C_s2, C_m1 and C_m2) using the Matlab software (MathWorks, Natick, MA, United States).

To calculate J_{max}, we used the electron transport limited expressions of V_p and V_c:

$$V_p = \frac{xJ_t}{2} \quad (12)$$

$$V_c = \frac{(1-x)J_t}{3\left(1 + \frac{7\gamma * O}{3C_s}\right)} \quad (13)$$

where x is a partitioning factor of electron transport, and J_t is the electron transport rate, given by:

$$J_t = \frac{I_2 + J_{\max} - \sqrt{(I_2 + J_{\max})^2 - 4\theta I_2 J_{\max}}}{2\theta} \quad (14)$$

where I_2 is the total absorbed irradiance, which is a function of the incident irradiance I, and θ is an empirical curvature factor.

By fitting equation (11) to equation (6), and equation (12) to equation (7), we obtained:

$$A = \frac{xJ_t}{2} - R_m - g_{bs} \times (C_s - C_m) \quad (15)$$

and

$$A = \frac{(1-x)J_t}{3\left(1 + \frac{7\gamma^* O}{3C_s}\right)} - R_d \quad (16)$$

C_m was obtained from equation (4). In equations (15) and (16), x and γ^* are constants at a given temperature, A, C_m and I (PPFD) are known values, and J_{max} and C_s are unknown entities. With two equations and two unknowns, and incorporating the single values of A and I (PPFD), we obtained the values for J_{max} and C_s.

Chlorophyll Fluorescence Measurements

Light adapted chlorophyll fluorescence was measured with a Li-Cor 6400 infrared gas analyzer while simultaneously measuring gas exchange, as described above. Steady-state fluorescence (F_s) was measured under actinic light. A saturating light pulse (∼8,000 μmol photons m^{-2} s^{-1}) was applied for 0.7 s to obtain the maximum fluorescence (F_m'). After removing the actinic light and applying 3 s of far-red light, the minimal fluorescence of the light-adapted state (F_o') was obtained. The quantum efficiency of PSII (Φ_{PSII}) and J_t were calculated using equations (17) and (18), respectively, following Genty et al. (1989) and Li et al. (2009):

$$\phi_{PSII} = \frac{F'_m - F_s}{F'_m} \quad (17)$$

$$J_t = \frac{F'_m - F_s}{F'_m} \times PPFD \times 0.85 \times 0.5 \quad (18)$$

The central portion of the same leaf (∼70% leaf area) was chosen for measurement of dark-adapted and light-adapted chlorophyll fluorescence parameters using a Fluor imager (CF Imager; Technologia Ltd., Colchester, United Kingdom). The minimum and maximum chlorophyll fluorescence (F_o and F_m, respectively) values were determined after full dark adaptation for at least 30 min. F_s, F_m', and F_o' were obtained as described above. The maximum quantum efficiency of PSII (F_v/F_m) was calculated using equation (32) of Genty et al. (1989):

$$F_v/F_m = \frac{F_m - F_o}{F_m} \quad (19)$$

Photochemical quenching (qL) was calculated using equation (20) and non-photochemical quenching (NPQ) was calculated using equation (21), following Kramer et al. (2004).

$$qL = \frac{F'_o}{F_s} \times \frac{F'_m - F_s}{F'_m - F'_o} \quad (20)$$

$$NPQ = \frac{F_m - F'_m}{F'_m} \quad (21)$$

Calculating Electron Flux to the Photosynthetic Carbon Reduction Cycle [Je(PCR)], and Electron Flux to the Photorespiratory Carbon Oxidation Cycle [Je(PCO)]

The J_t in the photosynthetic carbon reduction and photorespiratory carbon oxidation cycles were expressed as

follows (Zhou et al., 2004):

$$Je\,(PCR) = 4 \times \nu_c = 4 \times \frac{A + R_d}{1 - \frac{\Gamma*}{C_i}} \quad (22)$$

$$Je\,(PCO) = 4 \times \nu_o \quad (23)$$

Determination of Free NH_4^+ and Soluble Sugar Concentrations

The free NH_4^+ in plant tissues was determined according to Balkos et al. (2010) with some modifications. Briefly, plant tissues were desorbed in 10 mM $CaSO_4$ for 5 min, and then rinsed with deionized water to remove any extracellular NH_4^+. Approximately 0.5 g of fresh material was homogenized with liquid nitrogen; NH_4^+ was then extracted in 5 ml of 10 mM formic acid. Supernatants were collected after centrifugation at 10,000 *g* (4°C) for 15 min, transferred to 5-ml polypropylene tubes after filtration through 0.45-μm organic ultra-filtration membranes, and re-centrifuged at 50,000 *g* (4°C) for 10 min. An *O*-phthalaldehyde (OPA) reagent was prepared by combining 200 mM potassium phosphate buffer (equimolar amounts of potassium dihydrogen phosphate and potassium monohydrogen phosphate), 3.75 mM OPA, and 2 mM 2-mercaptoethanol (v/v/v = 1:1:1). Prior to adding 2-mercaptoethanol, the pH was adjusted to 7.0 using 1 M NaOH, and the solution was then filtered through two layers of filter paper. A 10-μl aliquot of tissue extract was mixed with 3 ml of OPA reagent. The color was developed in darkness at 25°C for 30 min before carrying out absorbance measurements at 410 nm using a spectrophotometer (model UV-2401, Shimadzu Corp., Kyoto, Japan).

Soluble sugar concentrations were measured following the method of Wang et al. (2016a). Dry powdered shoot and root samples (0.5 g) were extracted in 80% (v/v) ethanol at 80°C for 30 min. The extracts were later centrifuged at 3000 *g* for 10 min and the supernatants were collected. This extraction procedure was repeated three times to ensure all soluble sugars were extracted. The supernatants were evaporated on china dishes in a hot water bath. Residues were then re-dissolved in 1-3 ml of distilled water and filtered through 0.4-μm film to assay soluble sugars. Concentrations of soluble sugar were measured using the anthrone method. Anthrone sulfuric acid (5 ml) solution (75% v/v) was added to 0.1 ml of supernatant and heated to 90°C for 15 min. Absorbance at 620 nm was measured using a spectrophotometer (model UV-2401, Shimadzu Corp., Kyoto, Japan).

Statistical Analysis

We found significant effects of N forms (Mix-N, NN, AN) and CO_2 levels on the measured parameters in wheat and maize using the two-way ANOVA ($P < 0.05$, $n = 3$). Significant pairwise differences between means were identified with Dunnett's multiple comparisons test ($P < 0.05$). The proportion of variation (%) explainable by each factor was estimated as the total sums of squares. Calculations were performed with SPSS software (SPSS, Inc., Chicago, United States). Graphs were plotted using SigmaPlot 10.0 software (Systat Software, Inc., Chicago, IL, United States).

RESULTS

Dry Biomass, Leaf Area and Free NH_4^+

Compared with the Mix-N treatment, AN significantly reduced the shoot and root biomass of both wheat and maize plants (**Figure 2**). However, with increasing CO_2 concentration, wheat shoot and root biomass increased significantly, although these biomass parameters did not differ significantly according to CO_2 levels in maize. Shoot biomass in wheat under AN was reduced by 38%, 27%, and 14% at CO_2 concentrations of 280, 400 and 550 μmol mol^{-1}, respectively (in comparison with the Mix-N treatment). The decreases were larger in maize (46%, 44% and 44% at CO_2 concentrations of 280, 400, or 550 μmol mol^{-1}, respectively) (**Figure 2**). The AN treatment reduced the total leaf area, where the reduction was again greater in maize than in wheat. With increasing CO_2 concentrations, the total foliage area of wheat increased significantly, but this was not the case for maize. Free NH_4^+ concentrations did not differ significantly in either species between the Mix-N and NN treatments with increasing CO_2 concentration (**Figure 2**) whereas, in comparison with the other two treatments, the AN increased free NH_4^+ in shoots and roots. The concentration of free NH_4^+ decreased significantly with increasing CO_2 concentration in wheat, but not in maize.

Only the N form had significant effects on maize shoot dry biomass; in wheat, the N form, CO_2 level, and their interaction significantly affected shoot biomass (**Table 1**). Changes in CO_2 levels caused a higher proportion of the variance in wheat shoot dry biomass than did changes in N form. Total leaf area in the two species was significantly affected by both N form and CO_2 level. N form explained a larger proportion of the variance in total leaf area in both species. Alterations in CO_2 levels explained a much greater proportion of the variance in total leaf area variation in wheat (38%) than in maize (0.5%). In both species, the quantity of free NH_4^+ in shoots and roots differed significantly according to the form of N supplied. Interestingly, the CO_2 level had significant effects on free NH_4^+ in tissues of wheat, but not in maize.

Photosynthesis and Its Related Parameters

Compared with Mix-N and NN, AN treatment reduced the net photosynthetic rate (P_n) of both maize and wheat, although the effect was greater in the former species (**Figure 3**). The P_n of wheat plants growing under 550 μmol CO_2 mol^{-1} was significantly higher than that of plants grown under 280 and 400 μmol CO_2 mol^{-1}. Conversely, the P_n of maize plants under AN did not differ significantly according to the CO_2 level. On day 21 of the experiment, the P_n of maize under AN was reduced in comparison with those under the Mix-N treatment, by 34%, 32% and 32% at CO_2 concentrations of 280, 400, and 550 μmol mol^{-1}, respectively. The respective reductions in wheat were 30%, 27 % and 19%.

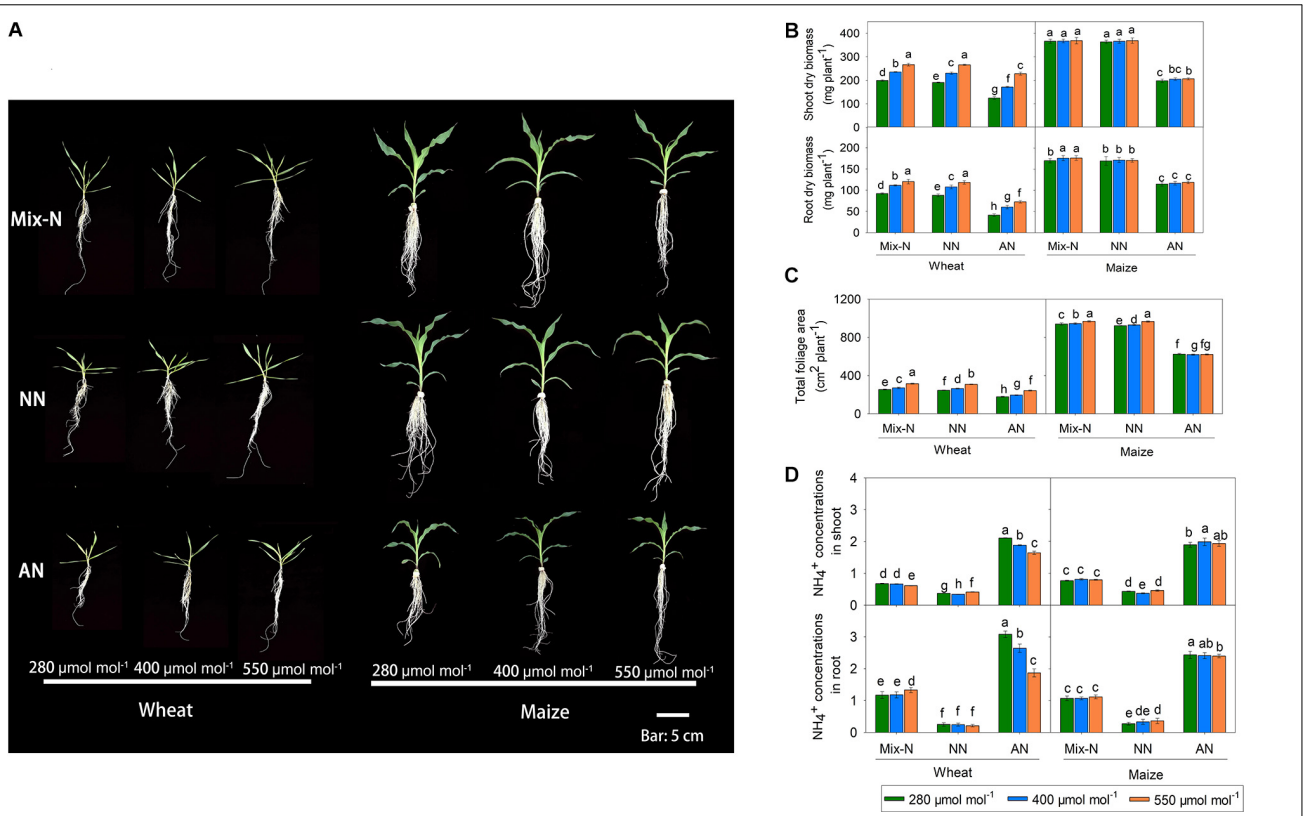

FIGURE 2 | Effects of CO_2 levels on shoot and root biomass (**A,B**, mg DW plant^{-1}), total foliage area (**C**, cm^2 plant^{-1}) and free NH_4^+ (**D**, μg g^{-1} FW) of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources. Data are means ± SE (n = 3); Lower-case letters indicate a significant difference at $p < 0.05$.

TABLE 1 | *F*-values in two-way ANOVA analysis of biomass, total foliage area and free NH_4^+ in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources.

Species	N-form	CO_2 level	Shoot dry weight		Root dry weight		Total foliage area		Free NH_4^+ in shoot		Free NH_4^+ in root	
Species	Source	df	Squares sum (×10^3)	*F*-value	Squares sum (×10^2)	*F*-value	Squares sum (×10^3)	*F*-value	Squares sum	*F*-value (×10^2)	Squares sum	*F*-value (×10^2)
Wheat	N	2	38.8(38.1)	2758**	279.8(76.5)	1822**	61(61.2)	1824**	23.09(97.1)	337.6**	47.6(91.0)	115.0**
	CO_2	2	60.5(59.4)	4302**	82.8(22.6)	539**	38(38.1)	1134**	0.24(1.0)	3.5**	1.2(5.3)	2.9**
	N × CO_2	4	2.3(2.3)	83**	0.3(0.1)	1 ns	0 (0.0)	1 ns	0.40(1.9)	3.3**	3.4(15.1)	4.1**
	Error	40	0.3(0.3)		3.1(0.8)		1(0.7)		0.01(0.1)		0.1(0.4)	
Maize	N	2	321.2(99.3)	3445.6**	372.4(97.9)	1279**	1258(99.2)	42843**	22.50(99.3)	48.0**	40.0(99.6)	71.9**
	CO_2	2	0.3(0.08)	2.7 ns	1.6(0.4)	5**	6(0.5)	197**	0.01(0.0)	0.0 ns	0.0(0.0)	0.0
	N × CO_2	4	0.1(0.03)	0.5 ns	0.6(0.1)	1 ns	4(0.3)	61**	0.04(0.2)	0.1**	0.0(0.2)	0.0
	Error	40	1.9(0.58)		5.8(1.5)		1(0.0)		0.09(0.4)		0.1(0.5)	

*N refers to the three nitrogen-forms effect; CO_2 refers to the three CO_2 levels effect; N × CO_2 refers to nitrogen-form × CO_2 levels interaction effects. Values in brackets following the sum of squares for variables indicate the percentage (%) of given variation to total variation. ** indicate significant at 0.01 level, respectively. df refers to the degree of freedom. ns means no significant difference.*

The g_s values did not differ significantly by CO_2 concentration, but were significantly reduced by AN treatment compared with the other two treatments, in both species (**Figure 3**). C_i increased significantly with increasing CO_2 concentration in wheat, but not in maize. The AN treatment significantly increased C_i in both species in comparison with the other two treatments. On day 21 of the experiment, we found no significant differences in C_s according to either the treatment or CO_2 concentration. V_{pmax} did not vary significantly by CO_2 concentration, but was significantly reduced under AN in comparison with the other two treatments.

The V_{cmax} and J_{max} of wheat increased significantly with increasing CO_2 concentration within the same level of the N form factor, but this was not the case for maize (**Figure 3**).

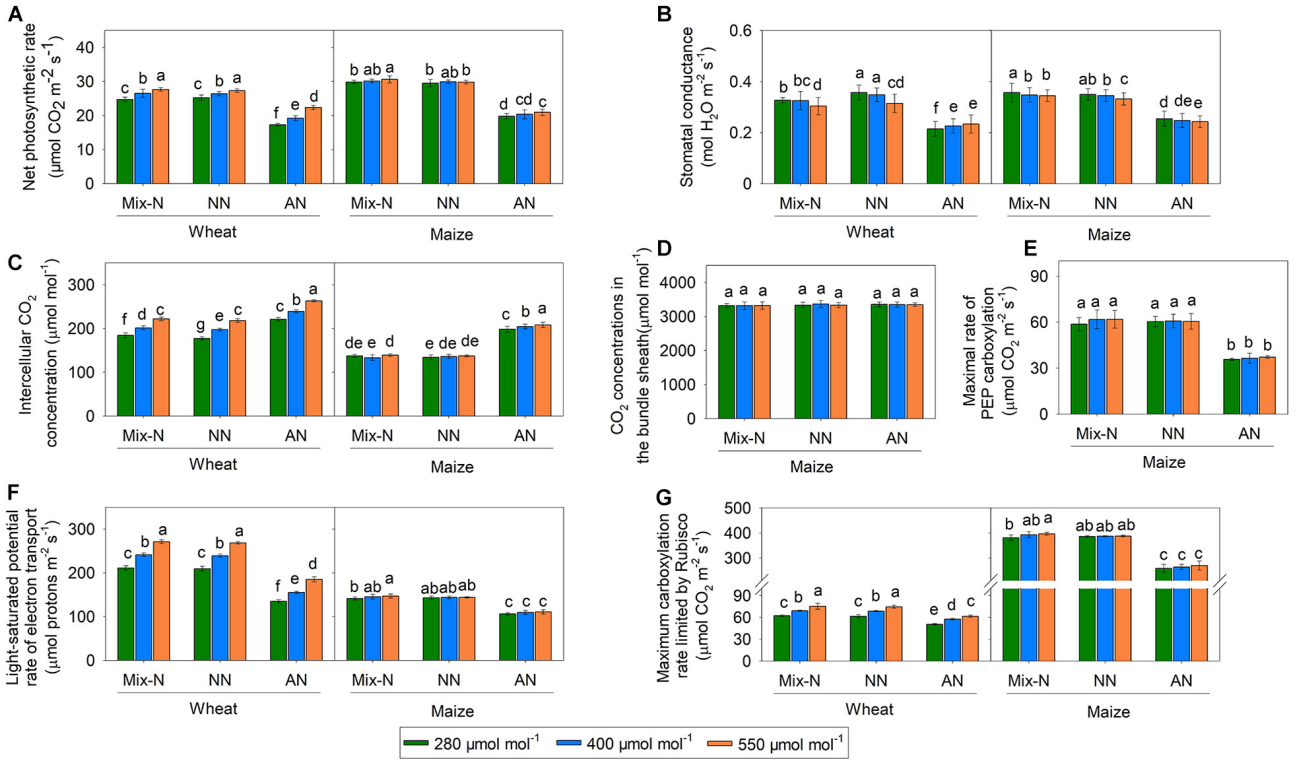

FIGURE 3 | Effects of CO_2 levels on P_n (**A**, μmol CO_2 m^{-2} s^{-1}), g_s (**B**, mol H_2O m^{-2} s^{-1}), C_i (**C**, μmol mol^{-1}), C_s (**D**, μmol mol^{-1}), V_{cmax} (**E**, μmol CO_2 m^{-2} s^{-1}), V_{pmax} (**F**, μmol CO_2 m^{-2} s^{-1}), and J_{max} (**G**, μmol protons m^{-2} s^{-1}) in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources. Data are means ± SE (n = 3); Lower-case letters indicate a significant difference at $p < 0.05$.

In comparison with the other two treatments, AN reduced V_{cmax} and J_{max} in both species. The reduction in V_{cmax} associated with AN was larger in maize than in wheat, while the reduction in J_{max} was smaller in maize than in wheat. On day 21 of the experiment, AN reduced V_{cmax} in maize in comparison with Mix-N, by 32%, 33% and 32% at CO_2 concentrations of 280, 400, and 550 μmol mol^{-1}, respectively. The respective reductions in wheat were 19%, 17%, and 18%. Moreover, AN reduced the J_{max} of maize in comparison with Mix-N, by 25%, 25%, and 24% at CO_2 concentrations of 280, 400, and 550 μmol mol^{-1}, respectively. The respective reductions in wheat were 36%, 36%, and 32%.

The P_n, g_s, C_i, V_{cmax} and J_{max} varied significantly according to both the N treatment type and the CO_2 level. The N form accounted for a larger proportion of the variance in these parameters in both species (**Table 2**). The effect of CO_2 level was much greater in wheat than in maize. The V_{pmax} of maize was significantly affected only by the N form. The C_s in maize was not affected by the N form, CO_2 level, or their interaction, nor by the interaction between pH and the N form.

Electron Transport Parameters

Under Mix-N and NN, the F_v/F_m, Φ_{PSII} and qL values of the two species did not vary significantly across different CO_2 concentration (**Figures 4A–C**). The values of these parameters decreased with increasing CO_2 concentration in maize plants under AN, but increased in wheat plants as CO_2 concentrations rose. NPQ increased significantly under AN in comparison with the other treatments, but did not differ significantly by CO_2 level (**Figure 4D**). The F_v/F_m, Φ_{PSII} and qL values of wheat varied significantly by both N form and CO_2 concentration, as observed for Φ_{PSII} and qL in maize (but not for Fv/Fm). N form explained a larger proportion of the variance in these parameters in both species than CO_2 concentration (**Table 3**). CO_2 level accounted for a larger proportion of the variance in electron transport in wheat (8.9% for F_v/F_m, 5.1% for Φ_{PSII} and 5.5% for qL) than in maize (0.0% for F_v/F_m, 0.5% for Φ_{PSII} and 0.4% for qL).

Under Mix-N and NN, the values of J_t for both species did not differ significantly across different CO_2 concentration (**Figure 5A**). However, while the values of J_t for wheat plants under AN increased significantly with increasing CO_2 concentration, this was not the case for maize. On day 21 of the experiment, AN reduced the J_t values to below those of plants under Mix-N, by 31%, 32% and 32% in maize at CO_2 concentrations of 280, 400, or 550 μmol mol^{-1}, respectively. The respective reductions in wheat were 38%, 30%, and 21%. The *Je(PCR)* values of wheat were higher under CO_2 concentrations of 400 and 550 μmol mol^{-1} than under a concentration of 280 μmol mol^{-1}. CO_2 level had no significant effect on the *Je(PCR)* values of maize (**Figure 5B**). In comparison with the other treatments, AN significantly reduced *Je(PCR)* in both species. Under AN, the *Je(PCR)* values of wheat increased

TABLE 2 | *F*-values in two-way ANOVA analysis of P_n, g_s, C_i, C_s, V_{cmax}, V_{pmax}, and J_{max} in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources.

Species	N-form	CO_2 level	P_n		g_s		C_i		C_s		V_{cmax}		V_{pmax}		J_{max}	
Species	**Source**	**df**	**Squares sum ($\times 10^3$)**	***F*-value**	**Squares sum ($\times 10^{-3}$)**	***F*-value**	**Squares sum ($\times 10^3$)**	***F*-value**	**Squares sum ($\times 10^2$)**	***F*-value**	**Squares sum ($\times 10^3$)**	***F*-value**	**Squares sum ($\times 10^2$)**	***F*-value**	**Squares sum ($\times 10^3$)**	***F*-value**
Wheat	N	2	532(80.7)	1217**	135(91.2)	732**	205.0(57.7)	2758**			1.75(53.7)	273**			80.1(73.0)	2290**
	CO_2	2	102(15.4)	232**	3(2.0)	16**	145.3(40.9)	4302**			1.38 (42.1)	214**			28.7(26.1)	820**
	N × CO_2	4	17(2.5)	18*	6(4.1)	16**	0.4(0.1)	83**			0.01(0.3)	1 ns			0.2(0.2)	3*
	Error	40	9(1.3)		4(2.7)		4.7(1.3)				0.13(3.9)				0.7(0.6)	
Maize	N	2	1108(98.3)	1647**	116(96.7)	1113**	546.3(98.4)	2396**	108(3.4)	0.722ns	184.7(97.0)	826**	70.5(92.1)	253.9**	14.9(94.9)	475**
	CO_2	2	5(0.4)	7**	2(1.7)	16**	2.4(0.4)	10**	10(0.3)	0.066ns	0.9(0.5)	4*	0.3(0.4)	1.0 ns	0.1(0.9)	4*
	N × CO_2	4	1(0.1)	1 ns	0 (0.0)	1 ns	2.0(0.4)	4**	28(0.9)	0.094ns	0.3(0.2)	1 ns	0.2(0.2)	0.3 ns	0.0(0.3)	1 ns
	Error	40	13(1.2)		2(1.7)		4.6(0.8)		2987(95.3)		4.5(2.3)		5.5(7.3)		0.6(4.0)	

*N refers to the three nitrogen-forms effect; CO_2 refers to the three CO_2 levels effect; N × CO_2 refers to nitrogen-form × CO_2 levels interaction effects. Values in brackets following the sum of squares for variables indicate the percentage (%) of given variation to total variation. * and ** indicate significant at 0.05 and 0.01 level, respectively. df refers to the degree of freedom. ns means no significant difference.*

significantly with increasing CO_2 concentration, but this was not the case for maize. When compared to other N form treatments, AN significantly reduced *Je(PCO)* in both species (**Figure 5C**) on day 21 of the experiment. The *Je(PCR)/Je(PCO)* ratio increased significantly with increasing CO_2 concentration in wheat. The ratio in maize was unaffected by either CO_2 level or N form (**Figure 5D**).

J_t, *Je(PCR) Je(PCO)* and the *Je(PCR)/Je(PCO)* ratio were significantly affected by N form and CO_2 level in both species. N form explained a larger proportion of the variance in these parameters in both species (**Table 4**). In wheat, 40% of the variance in the *Je(PCR)/Je(PCO)* ratio could be attributed to CO_2 level, but this factor accounted for only 2.9% of the variance in maize (**Table 4**).

Soluble Sugars

In comparison with the other N form treatments, AN markedly reduced the soluble sugar concentration in both species. Soluble sugar levels in the shoots and roots of wheat increased with increasing CO_2 concentration, but this was not the case in maize (**Figure 6**).

DISCUSSION

Increased Atmospheric CO_2 Concentrations Offset NH_4^+-Linked Stress in C_3 Wheat but Not in C_4 Maize

Unlike field or pot experiments, hydroponic experiments remove any potential complex interaction between ions and soil particles that might affect nutrient availability, and thus plant growth and development (Conn et al., 2013; Nguyen et al., 2016). We harnessed the hydroponic approach to study the responses of C_3 wheat and C_4 maize to different N forms and three levels of CO_2 concentrations. In previous studies, the most obvious effect of AN was reduced biomass production (Britto and Kronzucker, 2002; Li et al., 2011; Wang et al., 2016b, 2019). In the present study, the Mix-N and NO_3^--fed C_3 wheat plants produced more dry biomass when the corresponding C_a concentration was increased, but this was not the case for C_4 maize, corroborating the findings of Leakey et al. (2006). When C_a was low, the leaf-free NH_4^+ content was highest in C_4 maize with NH_4^+ as the sole N source (**Figure 2**); concomitantly under these conditions, we recorded the lowest P_n values for maize (**Figure 3**).

In C_3 wheat, AN-induced photosynthetic inhibition was ameliorated by increasing the C_a concentration, but this effect was insignificant in C_4 maize (**Figure 3**). Franks et al. (2013) found that change in C_a concentration changes the rates of carboxylation by Rubisco (in C_3 plants) and PEP carboxylase (in C_4 plants); each of these enzymes has a crucial limiting step in the photosynthetic pathway. An initial increase in P_n (less pronounced in C_4 plants) at or above the ambient C_a concentration occurs because of the unique CO_2-concentrating mechanism associated with C_4 photosynthesis (Ghannoum et al., 1997; von Caemmerer and Farquhar, 1999). In our study, we

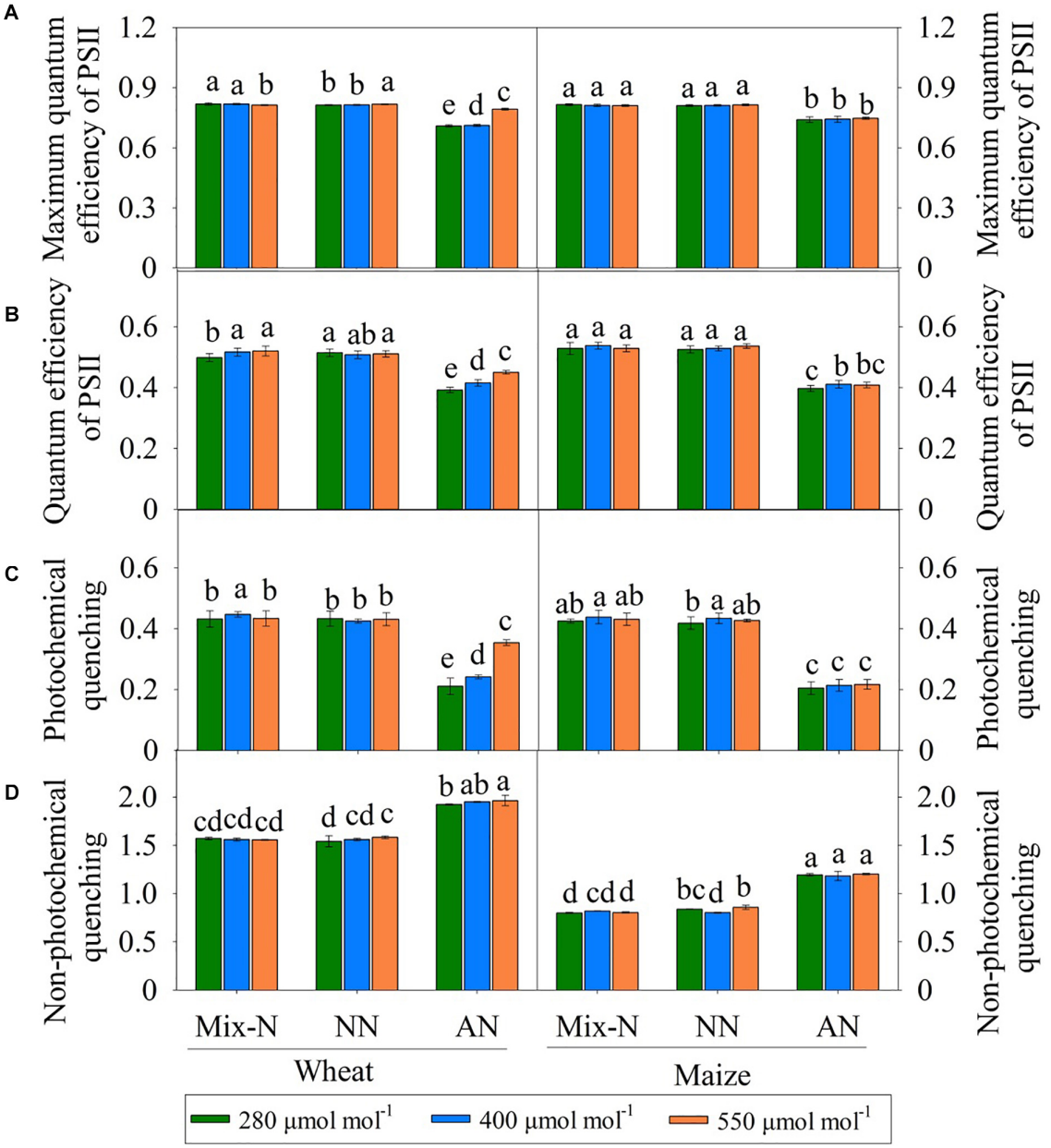

FIGURE 4 | Effects of different CO_2 levels on F_v/F_m **(A)**, Φ_{PSII} **(B)**, NPQ **(C)**, and *qL* **(D)** in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources. Data are means ± SE (n = 3); Lower-case letters indicate a significant difference at $p < 0.05$.

further explored (i) the potential limiting factors of P_n when C_3 and C_4 plants were grown under condition of NH_4^+-N nutrition, and (ii) the way in which changes in C_a concentration affected the potential NH_4^+ tolerance of C_3 and C_4 plants.

Impaired Electron Transfer Associated With NH_4^+ Inhibited Photosynthesis

Under atmospheric CO_2 conditions, the carboxylation ability of Rubisco is the key factor limiting C_3 photosynthesis (Li et al., 2009; Carmo-Silva et al., 2015). V_{cmax}, which represents the apparent Rubisco activity *in vivo* (Long and Bernacchi, 2003), increases with increasing CO_2 concentration (Jordan and Ogren, 1984). We found that both wheat and maize plants had lower g_s and V_{cmax} values under AN than under the other two N treatments, but C_i values were elevated under AN (**Figure 3**). Early CO_2 enrichment experiments using crops and tree saplings demonstrated that g_s was generally reduced by elevated CO_2 concentrations; we noted a similar phenomenon in wheat (**Figure 3**) (Yong et al., 1997; Medlyn et al., 2001). In contrast, the C_i and C_s of maize changed less with increases in C_a, regardless of the type of N nutrition. Li et al. (2015) suggested

TABLE 3 | *F*-values in two-way ANOVA analysis of F_v/F_m, Φ_{PSII}, NPQ and *qL* in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources.

Species	Source	df	*Fv/Fm*		Φ_{PSII}		NPQ		*qL*	
			Squares sum ($\times 10^{-3}$)	*F*-value	Squares sum ($\times 10^{-3}$)	*F*-value	Squares sum ($\times 10^{-3}$)	*F*-value	Squares sum ($\times 10^{-3}$)	*F*-value
Wheat	N	2	73(72.3)	11450**	102(86.4)	510**	1768(98.1)	1485**	326(81.7)	1753**
	CO_2	2	9(8.9)	1369**	6(5.1)	29**	5(0.2)	4*	22(5.5)	117**
	N × CO_2	4	19(18.8)	1478**	6(5.1)	15**	6(0.5)	3*	47(11.8)	127**
	Error	40	0(0.0)		4(3.4)		24(0.9)		4(1.0)	
Maize	N	2	57(96.6)	478**	189(96.7)	1013**	1671(98.4)	2249**	564(98.8)	2277**
	CO_2	2	0(0.0)	0 ns	1(0.5)	4*	4(0.2)	5*	2(0.4)	6**
	N × CO_2	4	0(0.0)	1 ns	1(0.5)	2 ns	8(0.5)	6**	0(0.0)	1 ns
	Error	40	2(3.4)		4(2.1)		15(0.9)		5(0.9)	

*N refers to the three nitrogen-forms effect; CO_2 refers to the three CO_2 levels effect; N × CO_2 refers to nitrogen-form × CO_2 levels interaction effects. Values in brackets following the sum of squares for variables indicate the percentage (%) of given variation to total variation. * and ** indicate significant at 0.05 and 0.01 level, respectively. df refers to the degree of freedom. ns means no significant difference.*

that the increases in C_i may result from decreases in the rate of the photosynthetic dark CO_2 reduction when g_s is reduced in C_3 plants.

Under ambient conditions, 44% of the absorbed light at peak PPFD was used for photosynthetic electron transport (25% for CO_2 fixation, 19% for photorespiration), and the remaining 56% was dissipated by chlorophyll fluorescence and thermal energy generation (Demmig-Adams and Adams, 1992). The balance between photosynthetic electron harvesting and transport within the chloroplasts is important for CO_2 assimilation based on the Calvin cycle (Demmig-Adams et al., 1989; Fryer et al., 1998; Shikanai, 2011). We found that *Fv/Fm*, Φ_{PSII}, *qL* and J_t were reduced under conditions of NH_4^+ nutrition (**Figures 4A–C**, **5A**), indicating that the energy available for CO_2 assimilation was limited. Similar responses under conditions of NH_4^+ nutrition were reported by Johnson et al. (2011), where the photosynthetic electron transport chain was interrupted on the PSII side. The oxygen-evolving complex of PSII may be a direct target of NH_4^+, causing a marked decline in photosynthesis (Drath et al., 2008). We found significant reductions in J_{max}, Φ_{PSII} and J_t for both wheat and maize (**Figures 3**, **4B**, **5A**); the reductions for maize were especially marked, and led to deficiencies in NADPH and ATP availability for CO_2 assimilation (Gao et al., 2018). Cousins and Bloom (2003) suggested that NO_3^- assimilation increases linear electron transfer and alleviates the photosynthetic ATP limitation in maize. In NH_4^+-fed plants, the inadequate energy supply for CO_2 carboxylation may be a result of interruptions in the electron transport chain (Wang et al., 2019). With an impaired PSII, plants have a reduced capacity to dissipate excitation energy through *qL*, resulting in a surplus of light energy (Kim and Apel, 2013). We found that NPQ increased in both species (**Figure 4D**) via a process involving the scavenging of excess light energy through heat dissipation under conditions of NH_4^+ nutrition. This finding was consistent with a previous report showing that plants can dissipate excess excitation energy in the form of heat through NPQ when they encountered abiotic stresses (Demmig-Adams and Adams, 1992).

Higher C_a Enhanced CO_2 Assimilation, Which Provided Additional C Skeletons for NH_4^+ Assimilation in C_3 Plants

Under AN, the J_{max}, Φ_{PSII} and J_t values of wheat increased with increasing CO_2 concentration (**Figures 3**, **4B**, **5A**), indicating that the interruption in electron transport can be offset by higher CO_2 concentration; these parameters did not differ significantly for maize grown under different CO_2 levels. At low atmospheric CO_2 levels, Rubisco utilizes both CO_2 and O_2 (Edwards et al., 2010). The process of uptaking O_2 leads to photorespiration, resulting in net losses of ≤40% of photosynthetic carbon under present day CO_2 levels of 400 μmol mol^{-1} (Andrews and Lorimer, 1978; Sage, 2004; Bloom, 2015). C_4 photosynthesis suppresses photorespiration by concentrating CO_2 internally (Andrews and Lorimer, 1978; Ehleringer et al., 1991). Conversely, higher C_a increases the CO_2 assimilation of C_3 plants and thereby inhibiting photorespiration; C_4 plants do not respond in this way (Andrews and Lorimer, 1978). We found that the C_i and C_c values of wheat under AN increased significantly with increased C_a, leading to increases in *Je(PCR)*, whereas *Je(PCO)* did not change significantly (**Figure 5D**). As a result, the *Je(PCR)/Je(PCO)* ratio increased with increasing CO_2 concentration under AN (**Figure 5C**). These findings indicated that the electron flux to CO_2 assimilation was increased at higher CO_2 concentrations, which may compensate for the decrease in electron transport ability seen under AN, thereby sustaining carbon assimilation. In maize, there were no significant differences in C_i, C_s or *Je(PCR)/Je(PCO)* by CO_2 level on day 21 of the experiment (**Figures 2**, **5D**).

Bloom et al. (2010) and Bloom (2015) found that (i) elevated CO_2 inhibits nitrite (NO_2^-) transport into chloroplasts, (ii) the chloroplast stroma compete for reduced ferredoxin (Fdr), and (iii) elevated CO_2 levels decrease photorespiration, thereby inhibiting shoot NO_3^- assimilation in C_3 plants under elevated CO_2 concentrations. In contrast, the first carboxylation reaction in the C_4 carbon fixation pathway generates ample quantities of malate and NADH in the cytoplasm of mesophyll cells.

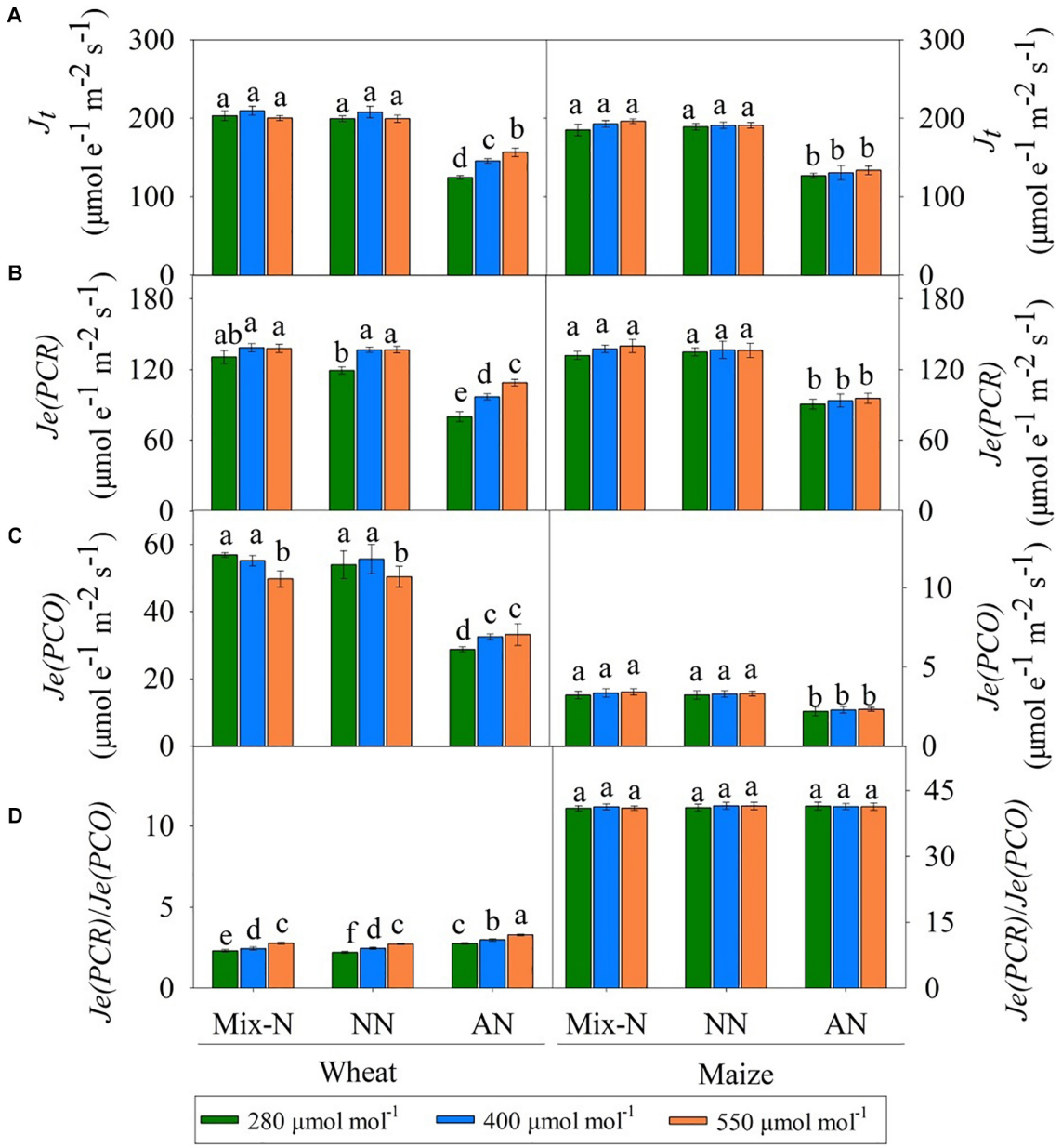

FIGURE 5 | Effects of different CO_2 levels on J_t **(A)**, *Je(PCR)* **(B)**, *Je(PCO)* **(C)**, and ratio of *Je(PCR)/ Je(PCO)* **(D)** in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources. Data are means ± SE (n = 3); Lower-case letters indicate a significant difference at $p < 0.05$.

This explains adequately the CO_2-independent shoot NO_3^- assimilation in C_4 plants (Bloom et al., 2010). However, since N assimilation occurs rapidly when NH_4^+ is the sole source of N nutrition, an adequate C skeleton supply for NH_4^+ assimilation is required to facilitate general physiological homoeostasis under elevated NH_4^+ concentrations (Ariz et al., 2013). Therefore, the carbohydrate status of plant tissues has an important role in the transition and adaptation to AN nutrition. A shortage of carbon assimilation for NH_4^+-form has been associated with a reduced level of soluble sugars in NH_4^+-grown plants (Setien et al., 2013). We found a significant decrease in the soluble sugar concentration in both species, especially in roots, under AN and ambient CO_2 conditions (**Figure 6**). With increasing C_a, an increase in soluble sugar concentration and a decrease in free NH_4^+ concentration occurred in wheat, possibly because of an increase in CO_2 photosynthetic capacity (Ariz et al., 2010, 2013). Therefore, in wheat, an increased P_n under AN, which was driven by elevated C_a levels, increased the supply of carbon skeleton for NH_4^+ assimilation, which in turn reduced the NH_4^+ concentrations and thereby ameliorating the NH_4^+ stress.

TABLE 4 | *F*-values in two-way ANOVA analysis of J_t, *Je(PCR)*, *Je(PCO),* and ratio of *Je(PCR)/ Je(PCO)* in newly expanded leaves of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources.

	Source	df	J_t		*Je(PCR)*		*Je(PCO)*		*Je(PCR)/Je(PCO)*	
Species			**Squares sum ($\times 10^3$)**	***F*-value**	**Squares sum ($\times 10^3$)**	***F*-value**	**Squares sum**	***F*-value**	**Squares sum**	***F*-value**
Wheat	N	2	44.5(90.5)	931**	17.6 (79.7)	719**	5885 (90.1)	363.3**	3.29(57.1)	454.2**
	CO_2	2	1.4(2.9)	30**	3.2 (14.5)	130**	104 (1.6)	6.4**	2.30 (40.0)	318.0**
	N × CO_2	4	2.3(4.6)	23**	0.8 (3.5)	15**	219 (3.4)	6.7**	0.22 (0.4)	1.5 ns
	Error	40	1.0(1.9)		0.5 (2.2)		323 (5.0)		0.15 (2.5)	
Maize	N	2	43.9 (96.5)	838**	22.2 (95.2)	529**	123 (90.5)	225.9**	0.97(6.1)	1.4 ns
	CO_2	2	0.4 (0.9)	7**	0.2 (0.9)	5**	0 (1.3)	3.3*	0.46 (2.9)	0.6 ns
	N × CO_2	4	0.1 (0.3)	1 ns	0.1 (0.3)	1 ns	0 (0.1)	0.1 ns	0.64 (4.1)	0.4 ns
	Error	40	1.0(2.3)		0.8 (3.6)		1 (8.0)		13.64 (86.8)	

*N refers to the three nitrogen-forms effect; CO_2 refers to the three CO_2 levels effect; N × CO_2 refers to nitrogen-form × CO_2 levels interaction effects. Values in brackets following the sum of squares for variables indicate the percentage (%) of given variation to total variation. * and ** indicate significant at 0.05 and 0.01 level, respectively. df refers to the degree of freedom. ns means no significant difference.*

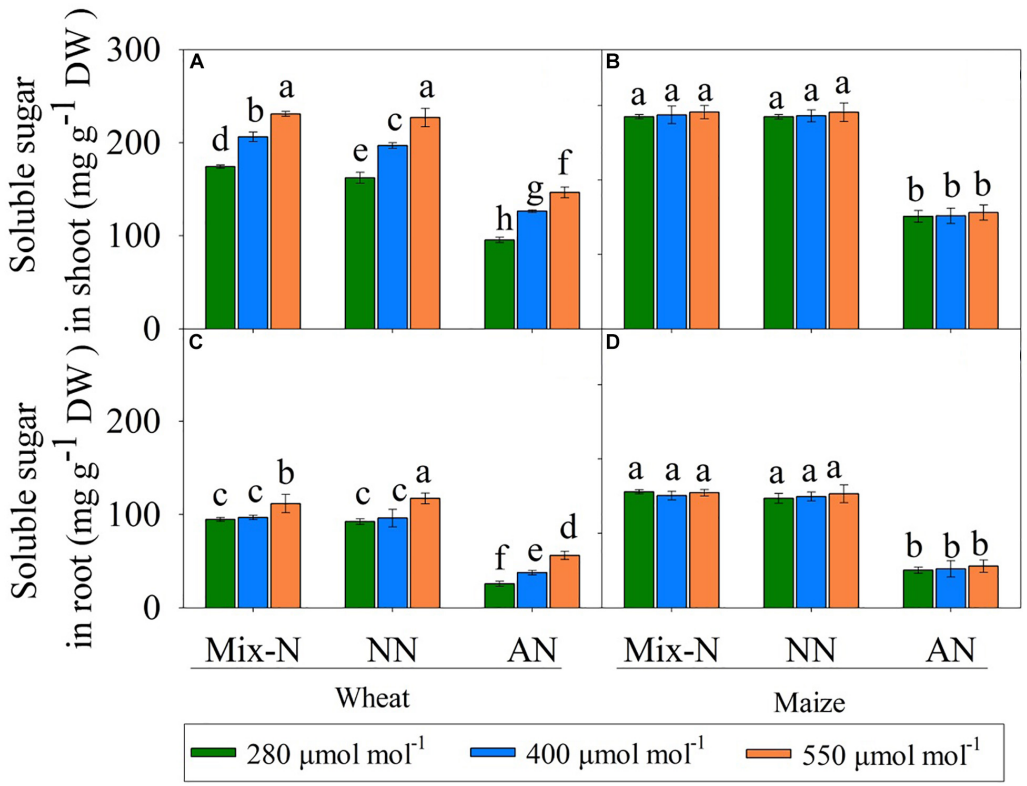

FIGURE 6 | Effects of different CO_2 levels on soluble sugar concentrations (mg g^{-1} DW) in shoots **(A,B)** and roots **(C,D)** of C_3 wheat and C_4 maize seedlings after 21 days of treatment with different N-sources. Data are means ± SE (n = 3); Lower-case letters indicate a significant difference at $p < 0.05$.

CONCLUSION

In conclusion, under ambient CO_2 conditions and AN nutrition, electron transport was reduced in both the C_3 wheat and C_4 maize plants, leading to a suppression of photosynthetic carbon assimilation. In wheat growing under elevated atmospheric CO_2 concentrations (C_a), increased C_i and C_c values improved electron flux to CO_2 assimilation rather than to photorespiration, thus sustaining photosynthesis and alleviating NH_4^+-induced stress. In contrast, elevated C_a had a negligible effect on C_i and C_s in maize and, consequently, minor effects on photosynthesis. Therefore, future increases in atmospheric C_a should provide C_3 plants with more opportunities to use NH_4^+ rather than relying on NO_3^- as a source of N fertilizers for crop production. Analyses using molecular biology and mutants to explain the possible physiological mechanisms in NH_4^+ tolerance of crop cultivars.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

FW and XH conceived the original screening and research plans. FW, JG, and XH supervised the experiments. FW performed most of the experiments, conceived the project, and wrote the article with salient contributions from all the authors in specific areas. FW, JG, JY, QW, JM, and XH supervised and completed the writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.537443/full#supplementary-material

REFERENCES

Anderson, L. J., Maherali, H., Johnson, H. B., Polley, H. W., and Jackson, R. B. (2001). Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C_3-C_4 grassland. *Global Change Biol.* 7, 693–707. doi: 10.1046/j.1354-1013.2001.00438.x

Andrews, T. J., and Lorimer, G. H. (1978). Photorespiration-still still unavoidable? *FEBS Lett.* 90, 1–9. doi: 10.1016/0014-5793(78)80286-5

Aranjuelo, I., Pardo, A., Biel, C., Save, R., Azcon-Bieto, J., and Nogues, S. (2009). Leaf carbon management in slow-growing plants exposed to elevated CO_2. *Global Change Biol.* 15, 97–109. doi: 10.1111/j.1365-2486.2008.01829.x

Ariz, I., Artola, E., Asensio, A. C., Cruchaga, S., Aparicio-Tejo, P. M., and Moran, J. F. (2011). High irradiance increases NH_4^+ tolerance in *Pisum sativum*: higher carbon and energy availability improve ion balance but not N assimilation. *J. Plant Physiol.* 168, 1009–1015. doi: 10.1016/j.jplph.2010.11.022

Ariz, I., Asensio, A. C., Zamarreno, A. M., Garcia-Mina, J. M., Aparicio-Tejo, P. M., and Moran, J. F. (2013). Changes in the C/N balance caused by increasing external ammonium concentrations are driven by carbon and energy availabilities during ammonium nutrition in pea plants: the key roles of asparagine synthetase and anaplerotic enzymes. *Physiol. Plant.* 148, 522–537. doi: 10.1111/j.1399-3054.2012.01712.x

Ariz, I., Esteban, R., García-Plazaola, J. I., Becerril, J. M., Aparicio-Tejo, P. M., and Moran, J. F. (2010). High irradiance induces photoprotective mechanisms and a positive effect on NH_4^+ stress in *Pisum sativum* L. *J. Plant Physiol.* 167, 1038–1045. doi: 10.1016/j.jplph.2010.02.014

Balkos, K. D., Britto, D. T., and Kronzucker, H. J. (2010). Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). *Plant Cell Environ.* 33, 23–34.

Bloom, A. J. (2015). Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. *Photosynth. Res.* 123, 117–128. doi: 10.1007/s11120-014-0056-y

Bloom, A. J., Burger, M., Asensio, J. S. R., and Cousins, A. B. (2010). Carbon dioxide enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*. *Science* 328, 899–903. doi: 10.1126/science.1186440

Bloom, A. J., Burger, M., Kimball, B. A., and Pinter, P. J. (2014). Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat. *Nat. Clim. Chang.* 4:477. doi: 10.1038/nclimate2183

Bloom, A. J., Smart, D. R., Nguyen, D. T., and Searles, P. S. (2002). Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proc. Natl. Acad. Sci.* U. S. A. 99, 1730–1735. doi: 10.1073/pnas.022627299

Britto, D. T., and Kronzucker, H. J. (2002). NH_4^+ toxicity in higher plants: a critical review. *J. Plant Physiol.* 159, 567–584. doi: 10.1078/0176-1617-0774

Carmo-Silva, E., Scales, J. C., Madgwick, P. J., and Parry, M. A. (2015). Optimizing Rubisco and its regulation for greater resource use efficiency. *Plant, Cell Environ.* 38, 1817–1832. doi: 10.1111/pce.12425

Conn, S. J., Hocking, B., Dayod, M., Xu, B., Athman, A., Henderson, S., et al. (2013). Protocol: optimising hydroponic growth systems for nutritional and physiological analysis of *Arabidopsis thaliana* and other plants. *Plant Methods* 9:4. doi: 10.1186/1746-4811-9-4

Coskun, D., Britto, D. T., Shi, W., and Kronzucker, H. J. (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nat. Plants* 3:17074.

Cousins, A. B., and Bloom, A. J. (2003). Influence of elevated CO_2 and nitrogen nutrition on photosynthesis and nitrate photossimilation in maize (*Zea mays* L.). *Plant Cell Environ.* 26, 1525–1530. doi: 10.1046/j.1365-3040.2003.01075.x

Demmig-Adams, B., and Adams, W. I. I. I. (1992). Photoprotection and other responses of plants to high light stress. *Ann. Rev. Plant Biol.* 43, 599–626. doi: 10.1146/annurev.pp.43.060192.003123

Demmig-Adams, B., Winter, K., Krüger, A., and Czygan, F. C. (1989). Light response of CO_2 assimilation, dissipation of excess excitation energy, and zeaxanthin content of sun and shade leaves. *Plant Physiol.* 90, 881–886. doi: 10.1104/pp.90.3.881

Dier, M., Meinen, R., Erbs, M., Kollhorst, L., Baillie, C. K., Kaufholdt, D., et al. (2018). Effects of free air carbon dioxide enrichment (FACE) on nitrogen assimilation and growth of winter wheat under nitrate and ammonium fertilization. *Global Change Biol.* 24, e40–e54.

Drath, M., Kloft, N., Batschauer, A., Marin, K., Novak, J., and Forchhammer, K. (2008). Ammonia triggers photodamage of photosystem II in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Plant Physiol.* 147, 206–215. doi: 10.1104/pp.108.117218

Edwards, E. J., Osborne, C. P., Strömberg, C. A., Smith, S. A., and Consortium, C. G. (2010). The origins of C_4 grasslands: integrating evolutionary and ecosystem science. *Science* 328, 587–591. doi: 10.1126/science.1177216

Ehleringer, J. R., Sage, R. F., Flanagan, L. B., and Pearcy, R. W. (1991). Climate change and the evolution of C_4 photosynthesis. *Trends Ecol. Evol.*. 6:95. doi: 10.1016/0169-5347(91)90183-x

Farquhar, G. D., von Caemmerer, ., and Berry, J. (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149, 78–90. doi: 10.1007/bf00386231

Fernández-Crespo, E., Camañes, G., and García-Agustín, P. (2012). Ammonium enhances resistance to salinity stress in citrus plants. *J. Plant Physiol.* 169, 1183–1191. doi: 10.1016/j.jplph.2012.04.011

Franks, P. J., Adams, M. A., Amthor, J. S., Barbour, M. M., Berry, J. A., Ellsworth, D. S., et al. (2013). Sensitivit.y of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. *New Phytol.* 197, 1077–1094. doi: 10.1111/nph.12104

Fryer, M. J., Andrews, J. R., Oxborough, K., Blowers, D. A., and Baker, N. R. (1998). Relationship between CO_2 assimilation, photosynthetic electron transport, and active O_2 metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiol.* 116, 571–580. doi: 10.1104/pp.116.2.571

Gao, J., Wang, F., Sun, J., Tian, Z., Hu, H., Jiang, S., et al. (2018). Enhanced Rubisco activation associated with maintenance of electron transport alleviates inhibition of photosynthesis under low nitrogen conditions in winter wheat seedlings. *J. Exp. Botany* 69, 5477–5488.

Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta Gen. Subj.* 990, 87–92. doi: 10.1016/s0304-4165(89)80016-9

Ghannoum, O., Caemmerer, S., Ziska, L. H., and Conroy, J. P. (2000). The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. *Plant Cell Environ.* 23, 931–942. doi: 10.1046/j.1365-3040.2000.00609.x

Ghannoum, O., von Caemmerer, S., Barlow, E. W., and Conroy, J. P. (1997). The effect of CO_2 enrichment and irradiance on the growth, morphology and gas exchange of a C_3 (*Panicum laxum*) and a C_4 (*Panicum antidotale*) grass. *Funct. Plant Biol.* 24, 407–407. doi: 10.1071/pp96077_co

Gong, P., Liang, L., and Zhang, Q. (2011). China must reduce fertilizer use too. *Nature* 473, 284–285. doi: 10.1038/473284e

Hatch, M. D., and Slack, C. R. (1966). Photosynthesis by sugar-cane leaves: a new carboxylation reaction and the pathway of sugar formation. *Biochem. J.* 101, 103–111. doi: 10.1042/bj1010103

IPCC (2007). *Agriculture: Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, and L. A. Meyer (Cambridge, New York, NY: Cambridge University Press), 497–540.

IPCC (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. (Cambridge, New York, NY: Cambridge University Press).

Johnson, M. P., Goral, T. K., Duffy, C. D., Brain, A. P., Mullineaux, C. W., and Ruban, A. V. (2011). Photoprotective energy dissipation involves the reorganization of photosystem II light-harvesting complexes in the grana membranes of spinach chloroplasts. *Plant Cell* 23, 1468–1479. doi: 10.1105/tpc.110.081646

Jordan, D. B., and Ogren, W. L. (1984). The CO_2/O_2 specificity of ribulose 1, 5-bisphosphate carboxylase/oxygenase. *Planta* 161, 308–313.

Kanemoto, K., Yamashita, Y., Ozawa, T., Imanishi, N., Nguyen, N. T., Suwa, R., et al. (2009). Photosynthetic acclimation to elevated CO_2 is dependent on N partitioning and transpiration in soybean. *Plant Sci.* 177, 398–403. doi: 10.1016/j.plantsci.2009.06.017

Kim, C., and Apel, K. (2013). Singlet oxygen-mediated signaling in plants: moving from flu to wild type reveals an increasing complexity. *Photosynth. Res.* 116, 455–464. doi: 10.1007/s11120-013-9876-4

Kramer, D. M., Johnson, G., Kiirats, O., and Edwards, G. E. (2004). New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth. Res.* 79:209. doi: 10.1023/b:pres.0000015391.99477.0d

Kühlbrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994). Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367, 614–621. doi: 10.1038/367614a0

Leakey, A. D., Uribelarrea, M., Ainsworth, E. A., Naidu, S. L., Rogers, A., Ort, D. R., et al. (2006). Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiol.* 140, 779–790. doi: 10.1104/pp.105.073957

Li, B., Shi, W., and Su, Y. (2011). The differing responses of two *Arabidopsis* ecotypes to ammonium are modulated by the photoperiod regime. *Acta Physiol. Plant.* 33, 325–334. doi: 10.1007/s11738-010-0551-5

Li, H., Wang, Y., Xiao, J., and Xu, K. (2015). Reduced photosynthetic dark reaction triggered by ABA application increases intercellular CO_2 concentration, generates H_2O_2 and promotes closure of stomata in ginger leaves. *Environ. Exp. Bot.* 113, 11–17. doi: 10.1016/j.envexpbot.2015.01.002

Li, Y., Gao, Y., Xu, X., Shen, Q., and Guo, S. (2009). Light-saturated photosynthetic rate in high-nitrogen rice (*Oryza sativa* L.) leaves is related to chloroplastic CO_2 concentration. *J. Exp. Bot.* 60, 2351–2360. doi: 10.1093/jxb/erp127

Lloyd, J., and Farquhar, G. D. (1996). The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. *Funct. Ecol.* 10, 4–32. doi: 10.2307/2390258

Long, S. P., and Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* 54, 2393–2401. doi: 10.1093/jxb/erg262

Medlyn, B., Barton, C. V. M., Broadmeadow, M. S. J., Ceulemans, R., De Angelis, P., Forstreuter, M., et al. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytol.* 149, 247–264. doi: 10.1046/j.1469-8137.2001.00028.x

Mehrer, I., and Mohr, H. (1989). Ammonium toxicity: description of the syndrome in *Sinapis alba* and the search for its causation. *Physiol. Plant.* 77, 545–554. doi: 10.1111/j.1399-3054.1989.tb05390.x

Miller, A. J., and Cramer, M. D. (2005). Root nitrogen acquisition and assimilation. *Plant Soil* 274, 1–36. doi: 10.1007/1-4020-4099-7_1

Nguyen, N. T., McInturf, S. A., and Mendoza-Cózatl, D. G. (2016). Hydroponics: a versatile system to study nutrient allocation and plant responses to nutrient availability and exposure to toxic elements. *J. Vis. Exp.* 113:e54317.

Onoda, Y., Hikosaka, K., and Hirose, T. (2004). Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Funct. Ecol.* 18, 419–425. doi: 10.1111/j.0269-8463.2004.00847.x

Owensby, C. E., Ham, J. M., Knapp, A. K., and Auen, L. M. (1999). Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. *Global Change Biol.* 5, 497–506. doi: 10.1046/j.1365-2486.1999.00245.x

Richter, J., and Roelcke, M. (2000). The N-cycle as determined by intensive agriculture–examples from central Europe and China. *Nutr. Cycl. Agroecosyst.* 57, 33–46.

Robredo, A., Pérez-López, U., Miranda-Apodaca, J., Lacuesta, M., Mena-Petite, A., and Muñoz-Rueda, A. (2011). Elevated CO_2 reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. *Environ. Exp. Bot.* 71, 399–408. doi: 10.1016/j.envexpbot.2011.02.011

Sage, R. F. (2004). The evolution of C4 photosynthesis. *New Phytol.* 161, 341–370.

Setien, I., Fuertes-Mendizabal, T., Gonzalez, A., Aparicio-Tejo, P. M., Gonzalez-Murua, C., Gonzalez-Moro, M. B., et al. (2013). High irradiance improves ammonium tolerance in wheat plants by increasing N assimilation. *J. Plant Physiol.* 170, 758–771. doi: 10.1016/j.jplph.2012.12.015

Shikanai, T. (2011). Regulation of photosynthetic electron transport. *Biochim. Biophys. Acta* 1807, 375–383.

von Caemmerer, S., and Farquhar, G. D. (1999). "The modelling of C_4 photosynthesis," in *The Biology of C_4 Photosynthesis*, ed. R. M. Sage (New York, NY: Academic Press)), 173–211.

Wang, F., Gao, J., Liu, Y., Tian, Z., Muhammad, A., Zhang, Y., et al. (2016a). Higher ammonium transamination capacity can alleviate glutamate inhibition on winter wheat (*Triticum aestivum* L.) root growth under high ammonium stress. *PLoS One* 11:e0160997. doi: 10.1371/journal.pone.0160997

Wang, F., Gao, J., Shi, S., He, X., and Dai, T. (2019). Impaired electron transfer accounts for the photosynthesis inhibition in wheat seedlings (*Triticum aestivum* L.) subjected to ammonium stress. *Physiol. Plant.* 167, 159–172. doi: 10.1111/ppl.12878

Wang, F., Gao, J., Tian, Z., Liu, Y., Abid, M., Jiang, D., et al. (2016b). Adaptation to rhizosphere acidification is a necessary prerequisite for wheat (*Triticum aestivum* L.) seedling resistance to ammonium stress. *Plant Physiol. Biochem.* 108, 447–455. doi: 10.1016/j.plaphy.2016.08.011

Xing, G., and Zhu, Z. (2000). An assessment of N loss from agricultural fields to the environment in China. *Nutr. Cycl. Agroecosyst.* 57, 67–73.

Xu, G., Fan, X., and Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Ann. Rev. Plant Biol.* 63, 153–182. doi: 10.1146/annurev-arplant-042811-105532

Yong, J. W. H., Letham, D. S., Wong, S. C., and Farquhar, G. D. (2010). Effects of root restriction on growth and associated cytokinin levels in cotton (*Gossypium hirsutum*). *Funct. Plant Biol.* 3, 974–984. doi: 10.1071/fp10009

Yong, J. W. H., Wong, S. C., and Farquhar, G. D. (1997). Stomatal responses to changes in vapour pressure difference between leaf and air. *Plant Cell Environ.* 20, 1213–1216. doi: 10.1046/j.1365-3040.1997.d01-27.x

Yong, J. W. H., Wong, S. C., Letham, D. S., Hocart, C. H., and Farquhar, G. D. (2000). Effects of elevated CO_2 and nitrogen nutrition on cytokinins in the xylem sap and leaves of cotton. *Plant Physiol.* 124, 767–780. doi: 10.1104/pp.124.2.767

Zhou, Y. H., Yu, J. Q., Huang, L. F., and Nogues, S. (2004). The relationship between CO_2 assimilation, photosynthetic electron transport and water-water cycle in chill-exposed cucumber leaves under low light and subsequent recovery. *Plant Cell Environ.* 27, 1503–1514. doi: 10.1111/j.1365-3040.2004.01255.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.