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Nitrate photo-assimilation in tomato leaves under short-term exposure to elevated carbon dioxide and low oxygen

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ABSTRACT

The role of photorespiration in the foliar assimilation of nitrate (NO_3^-) and carbon dioxide (CO_2) was investigated by measuring net CO_2 assimilation, net oxygen (O_2) evolution, and chlorophyll fluorescence in tomato leaves (*Lycopersicon esculentum*). The plants were grown under ambient CO_2 with ammonium nitrate (NH_4NO_3) as the nitrogen source, and then exposed to a CO_2 concentration of either 360 or 700 μmol mol^{-1}, an O_2 concentration of 21 or 2%, and either NO_3^- or NH_4^+ as the sole nitrogen source. The elevated CO_2 concentration stimulated net CO_2 assimilation under 21% O_2 for both nitrogen treatments, but not under 2% O_2. Under ambient CO_2 and O_2 conditions (i.e. 360 μmol mol^{-1} CO_2, 21% O_2), plants that received NO_3^- had 11–13% higher rates of net O_2 evolution and electron transport rate (estimated from chlorophyll fluorescence) than plants that received NH_4^+. Differences in net O_2 evolution and electron transport rate due to the nitrogen source were not observed at the elevated CO_2 concentration for the 21% O_2 treatment or at either CO_2 level for the 2% O_2 treatment. The assimilatory quotient (AQ) from gas exchange, the ratio of net CO_2 assimilation to net O_2 evolution, indicated more NO_3^- assimilation under ambient CO_2 and O_2 conditions than under the other treatments. When the AQ was derived from gross O_2 evolution rates estimated from chlorophyll fluorescence, no differences could be detected between the nitrogen treatments. The results suggest that short-term exposure to elevated atmospheric CO_2 decreases NO_3^- assimilation in tomato, and that photorespiration may help to support NO_3^- assimilation.

Key-words: *Lycopersicon esculentum*; assimilatory quotient; chlorophyll fluorescence; electron transport; oxygen exchange; photorespiration.

Abbreviations: AQ_F, ratio of net CO_2 assimilation to the gross rate of O_2 evolution estimated from chlorophyll fluorescence; AQ_G, ratio of net CO_2 assimilation to net O_2 evolution; ΔAQ, difference in AQ between NO_3^- and NH_4^+ treatments; J_{O2}, gross rate of O_2 evolution estimated from chlorophyll fluorescence; J_{PSII}, rate of linear electron transport through photosystem II; NPQ, non-photochemical quenching; q_P, photochemical quenching.

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INTRODUCTION

Foliar assimilation of nitrate (NO_3^-) and carbon dioxide (CO_2) interact through many complex pathways (Stitt & Krapp 1999; Paul & Foyer 2001). For example, the reduction of NO_3^- through nitrite (NO_2^-) to ammonia (NH_4^+) and its subsequent assimilation to glutamate via the glutamine synthetase/glutamate oxyglutarate aminotransferase cycle (GS/GOGAT) is an energy-demanding process requiring the transfer of 10 electrons compared to four electrons for the assimilation of CO_2 to carbohydrate (Turpin, Weger & Huppe 1997). Assimilation of NO_3^- and CO_2 may compete for reductant such as ferredoxin that is produced during photosynthetic electron transport (Bloom *et al*. 2002) because NO_2^- reduction and the GS/GOGAT cycle both reside within the chloroplast where CO_2 assimilation occurs. Additionally, the NH_4^+ used in leaf amino acid synthesis is derived both from the primary reduction of NO_3^- to NH_4^+ and from the NH_4^+ released during photorespiration (Keys *et al*. 1978; Novitskaya *et al*. 2002).

When C_3 plants such as barley, pea, and wheat receive NO_3^- rather than NH_4^+ as the sole nitrogen source, the rate of photosynthetic electron transport often increases (Bloom *et al*. 1989; De la Torre, Delgado & Lara 1991; Bloom *et al*. 2002). Using net O_2 evolution as the measure of electron transport, these increases tend to be light-dependent with the greatest differences found at high rather than low light. In tobacco, chlorophyll fluorescence measurements have indicated that the assimilation of NO_3^- can account for some percentage of electron transport even under low light conditions (Morcuende *et al*. 1998). Based on calculations from foliar C : N ratios, Foyer, Ferrario-Méry, & Noctor (2001) suggested that NO_3^- assimilation typically represents about 10% of photosynthetic electron flow although actual measurements of net O_2 evolution can lead to higher estimates for the same species (De la Torre *et al*. 1991).

In addition to CO_2 and NO_3^- assimilation, photorespiration expends a substantial amount of photosynthetic energy in C_3 plants. As a consequence of the specificity of RuBP carboxylase/oxygenase (Rubisco) for both CO_2 and O_2, elevated CO_2 or low O_2 concentrations reduce photorespiration and typically lessen the electron requirement per CO_2 assimilated (Stitt 1991). The substantial decrease in photo-

respiration under elevated CO_2 or low O_2 concentrations also removes the demand for photoreductant (Wingler *et al*. 2000). Thus, diminished rates of photorespiration may allow for more photosynthetic energy to be used in foliar NO_3^- assimilation (Matt *et al*. 2001).

The influence of elevated atmospheric CO_2 concentration on interactions between NO_3^- and CO_2 assimilation in wheat was recently examined in our laboratory (Bloom *et al*. 2002). Contrary to our expectation, short-term (i.e. hours) gas exchange measurements of shoots grown at ambient CO_2 levels indicated that exposure to elevated CO_2 decreased NO_3^- photo-assimilation. In longer-term experiments (i.e. days), wheat plants grown under elevated CO_2 had less foliar NO_3^- reductase and NO_2^- reductase activities, and less shoot protein than plants grown under ambient CO_2. Studies on *Plantago major* (Fonseca, Bowsher & Stulen 1997), *Nicotiana tabacum* (Geiger *et al*. 1999), *Nicotiana plumbaginifolia* (Ferrario-Mèry *et al*. 1997), and *Spinacia oleracea* (Kaiser *et al*. 2000) have also found that longer exposures (4 h to over 2 weeks) to elevated CO_2 can inhibit NO_3^- reductase activity in shoots.

In the following study, we conducted short-term experiments to examine the influence of both low oxygen and elevated atmospheric CO_2 concentrations on foliar NO_3^- photo-assimilation. Low oxygen conditions provide non-photorespiring conditions, and allow for a more direct assessment of photosynthetic energy demand by NO_3^- and CO_2 assimilation, particularly at ambient CO_2 concentrations, in which photorespiration is otherwise high. Nitrate assimilation was assessed using chlorophyll fluorescence measurements as well as measurements of photosynthetic gas exchange and changes in the assimilatory quotient (net CO_2 assimilation/net O_2 evolution). The assimilatory quotient, *AQ*, has been used successfully to assess NO_3^- assimilation in a number of species (Bloom *et al*. 1989; Cen, Turpin & Layzell 2001; Bloom *et al*. 2002).

MATERIALS AND METHODS

Plant cultivation

Tomato (*Lycopersicon esculentum* cv. Ailsa Craig) seeds were surface sterilized for 10 min in a 25% bleach solution, washed thoroughly with water, and germinated on moist cheesecloth that was suspended over aerated nutrient solution in 3 L opaque plastic containers. Two sets of five seeds were germinated each week to provide plants of the same age and size for the gas exchange measurements. The 3 L containers were placed in a controlled environment cuvette (Conviron, Winnipeg, Canada) set at 25 °C day/18 °C night with a 16 h photoperiod and ambient CO_2 concentration. The photosynthetic flux density (PFD) was 500–600 μmol m^{-2} s^{-1} at plant height. After 7 to 8 d, three to five seedlings were transferred to a larger, 19 L container. The aerated nutrient solution in both the 3 L and 19 L containers included 0.2 mM NH_4NO_3, 1 mM $CaSO_4$, 0.65 mM K_2HPO_4, 0.35 mM KH_2PO_4, 1 mM $MgSO_4$, 0.6 mM K_2SO_4, 0.01 g L^{-1} FeDPTA (sodium ferric diethylenetriaminepentaacet), and micronutrients (Epstein 1972). The nutrient solution in the 19 L containers was replenished after 7 d with a nutrient solution that was one-half strength of the original solution. Experiments were conducted on 17- to 19-day-old-plants that had two fully expanded leaves.

Laboratory protocol and experimental design

In the afternoon, approximately 15 h before an experiment, a plant was transferred from the growth chamber to the laboratory. The root system of this intact plant was sealed into an acrylic plastic cuvette in the laboratory by fitting a split rubber stopper around the stem. The root cuvette was filled with aerated nutrient solution and attached to the continuous flow nutrient system described by Nicoulaud & Bloom (1998). The nutrient solution contained either 0.2 mM KNO_3 or 0.2 mM NH_4Cl as the nitrogen source along with 1 mM $CaSO_4$ and 0.5 μM K_2HPO_4. The following morning, the terminal leaflet and two distal leaflets of the most recently expanded leaf were sealed into a single leaf gas exchange cuvette that held the leaf perpendicular below a 1000 W metal halide lamp (Wide-Lite, San Marcos, TX, USA). Copper–constantan thermocouples were placed on the undersides of the terminal leaflet and one of the distal leaflets to monitor leaf temperature. The leaf was allowed to equilibrate for 2 h at a PFD of 300 μmol m^{-2} s^{-1} and at the CO_2 and O_2 concentrations of a specific treatment. The PFD was then reduced to 100 μmol m^{-2} s^{-1} for 30 min. A leaf was therefore exposed to at least 2.5 h of a particular CO_2 and O_2 concentration before gas-exchange and chlorophyll fluorescence measurements were made. As a consequence, we focused on longer-term responses rather than the transients in NO_3^- assimilation that may occur after a change in conditions (Kaiser *et al*. 2000).

In addition to the two nitrogen treatments (0.2 mM KNO_3 or 0.2 mM NH_4Cl), a leaf was exposed to one of four atmospheric mixtures in the leaf cuvette: a CO_2 concentration of either 360 or 700 μmol mol^{-1} and an O_2 concentration of either 20 (2% O_2) or 210 mmol mol^{-1} (21% O_2). For the gas exchange measurements, six to nine replicates for each of the eight treatment combinations (nitrogen form $\times$ CO_2 $\times$ O_2) were performed. Chlorophyll fluorescence was measured simultaneously with gas exchange, but there was slightly less replication ($n = 5$) for the leaves measured at 21% O_2 due to equipment availability. Only one leaf was measured each day. The measurements were conducted at five different PFD levels (100, 300, 500, 800 and 1200 μmol m^{-2} s^{-1}) from the lowest to the highest PFD to minimize the influence of the preceding PFD on subsequent chlorophyll fluorescence measurements. The leaf was at each PFD level for about 30 min to allow for an accurate net O_2 evolution measurement using the custom O_2 analyser described below.

Gas exchange measurements

An open gas exchange system previously described by Bloom *et al*. (1989) monitored net CO_2 assimilation, net O_2

evolution, and transpiration using, respectively, a commercial non-dispersive infrared CO_2 analyser (Model VIA-500R; Horiba, Irvine, CA, USA), a custom-designed O_2 analyser, and relative humidity sensors (Vaisala, Helsinki, Finland). The custom O_2 analyser contains two cells of calcia-stabilized zirconium oxide ceramic similar to those found in an Applied Electrochemistry model N-37 M (Pittsburgh, PA, USA). Platinum electrodes are located on the inside and outside of each cell at one end. When heated to 752 ± 0.01 °C in an electric furnace, these cells become selectively permeable to O_2, and a 106-nV Nernst potential per μmol difference in O_2 concentration is generated between the two cells at a normal ambient O_2 background of 209 700 μmol mol^{-1} (or 20.97% O_2). As expected, the potential generated per oxygen concentration difference at 20 000 μmol mol^{-1} (or 2% O_2) was about 10 times greater than at 209 700 μmol mol^{-1}. In practice, this analyser can resolve O_2 concentration differences to better than 2 μmol mol^{-1} at 21 or 2% O_2 (Bloom *et al*. 1989).

Mass flow controllers (Tylan, Torrance, CA, USA) prepared the various gas mixtures. For the 21% O_2 experiments, 2% CO_2 in air from a compressed gas cylinder and CO_2-free air from a 100 L storage tank were mixed to obtain the 360 and 700 μmol mol^{-1} CO_2 concentrations. For the 2% O_2 experiments, the controllers mixed 2% CO_2 in nitrogen, pure oxygen, and pure nitrogen from three compressed gas cylinders. A pressure transducer (Validyne, North Ridge, CA, USA) monitored the gas flow through the leaf cuvette. The leaf cuvette was constructed from glass and Teflon-coated aluminium to minimize oxidative O_2 exchange. The gas flow in the gas exchange system was humidified in a water bubbler filled with glass beads and then partially dehumidified in a condenser cooled to 6 °C before reaching the leaf cuvette. The leaf vapour pressure deficit was maintained at approximately 10 mbar. Leaf and root solution temperatures were maintained at 25 and 20 °C, respectively.

The assimilatory quotient of gas exchange (AQ_G), the ratio of net CO_2 assimilation to net O_2 evolution, was used as a measure of foliar NO_3^- assimilation. Transfer of electrons to nitrate and to nitrite during NO_3^- assimilation increases O_2 evolution from the light-dependent reactions of photosynthesis, while CO_2 assimilation remains similar or decreases. Thus, leaves that are photo-assimilating NO_3^- should exhibit a lower *AQ*, and differences in the *AQ* between NO_3^- and NH_4^+ treatments (ΔAQ) should be correlated with NO_3^- assimilation.

Over half a century ago, Myers (1949) verified for algae that ΔAQ depended upon NO_3^- assimilation. We showed over a decade ago in a wild-type barley that ΔAQ reflected the difference between the NO_3^- absorbed and the NO_3^- accumulated (Bloom *et al*. 1989; Bloom, Sukrapanna & Warner 1992). Moreover, in barley mutants deficient in NO_3^- reductase, ΔAQs did not deviate from zero. More recently, Cen *et al*. (2001) documented that NO_3^- assimilation makes up about 74% of whole-plant reductant use in white lupin (*Lupinus alba*) and, thus, has a much greater effect upon ΔAQ than any other metabolic process. We have shown in wheat that ΔAQ correlates positively with nitrous oxide production (which depends on NO_3^- assimilation, Smart & Bloom 2001), leaf protein content, and nitrate reductase and nitrite reductase activities and that ΔAQ correlates negatively with accumulation of free NO_3^- (Bloom *et al*. 2002). In summary, all available data support that ΔAQ provides a real-time and continuous measure of NO_3^- assimilation.

Chlorophyll fluorescence measurements

A PAM 101 fluorometer (H. Walz GmbH, Effeltrich, Germany) equipped with a xenon-arc lamp to provide a saturating light pulse (10 000 μmol m^{-2} s^{-1} for 1 s) assessed chlorophyll fluorescence of the terminal leaflet. Steady-state fluorescence (F_s) and maximum fluorescence (F_m') were recorded at each PFD level on a chart recorder. The quantum efficiency of linear electron transport through photosystem II (ϕ_{PSII}) was calculated as $(F_m' - F_s)/F_m'$ according to the method of Genty, Briantais, & Baker (1989). The rate of linear electron transport through PSII (J_{PSII}) was then estimated as ($\phi_{PSII} \cdot \alpha \cdot 0.5$), where the coefficient of leaf absorptance of PFD (α) was assumed to be 0.85 and the factor 0.5 was used to account for the partitioning of energy between PSII and PSI. The assumption that $J_{PSII} = \phi_{PSII} \cdot 0.85 \cdot 0.5$ is standard (Maxwell & Johnson 2000).

The J_{PSII} is divided by 4, based on 4 e$^-$ transported per O_2 evolved, to estimate the gross rate of O_2 evolution (J_{O2}) (Edwards & Baker 1993). To calculate photochemical (q_P) and non-photochemical (NPQ) quenching at each PFD level, the maximum quantum efficiency of PSII [$F_V/F_m = (F_m - F_o)/F_m$] was measured in the dark before the experiment began. The q_P and NPQ were calculated as $(F_m' - F_s)/(F_m' - F_o)$ and $(F_m - F_m')/F_m$, respectively.

Using the simultaneous measurements of gas exchange and chlorophyll fluorescence, an AQ_F was determined using the ratio of net CO_2 assimilation to J_{O2}. This *AQ* is similar to that described earlier except that the O_2 evolution for AQ_F reflects a gross rate rather than a net rate.

Statistical analysis

A repeated measures analysis of variance was performed using the mixed procedure in SAS (PROC MIXED; SAS Institute, Cary, NC, USA) to investigate the effects of nitrogen form (N), CO_2 treatment, O_2 treatment, and PFD on the gas exchange and fluorescence parameters. The PFD was considered to be a repeated factor since each leaf was measured at all five levels of PFD. Natural log or square root transformations were used where appropriate to normalize the data for a given dependent variable. Effects of the treatments and their interactions were considered significant when $P = 0.05$ and are presented in Table 1.

RESULTS

Net CO_2 assimilation at the higher PFD levels was greater under elevated CO_2 (700 μmol mol^{-1}) than ambient CO_2 (360 μmol mol^{-1}) for both N treatments under normal

Table 1. Analyses of variance for gas exchange and chlorophyll fluorescence parameters of tomato leaves under two nitrogen sources (0.2 mM KNO_3, 0.2 mM NH_4Cl), two CO_2 concentrations (360 μmol mol^{-1}, 700 μmol mol^{-1}), two O_2 concentrations (2%, 21%), and at five different irradiance (PFD) levels

Source of variation	Net CO_2 uptake	Net O_2 evolution	AQ_G (CO_2/O_2)	J_{PSII}	AQ_F (CO_2/J_{O2})	NPQ	q_P
N							
O_2		**	**		**		**
$N \times O_2$							
CO_2	**	*			**	**	
$N \times CO_2$							
$O_2 \times CO_2$	*				**	*	
$N \times O_2 \times CO_2$			*				
PFD	**	**	**	**	**	**	**
$N \times PFD$							
$O_2 \times PFD$	**	**	**	**	**	**	**
$N \times O_2 \times PFD$							
$CO_2 \times PFD$	**	*	*				
$N \times CO_2 \times PFD$			**				
$O_2 \times CO_2 \times PFD$	*		**				
$N \times O_2 \times CO_2 \times PFD$						*	

Statistically significant main effects and interactions are shown as: $*P \leq 0.05$, $**P \leq 0.01$.

atmospheric O_2 (21%) (Fig. 1a). In contrast, net CO_2 assimilation was not enhanced by elevated CO_2 under 2% O_2 (Fig. 1b). Relative to normal atmospheric O_2, 2% O_2 stimulated net CO_2 assimilation under ambient CO_2 at low PFD, but no difference was apparent at high PFD. All of the above responses contributed to the three-way $O_2 \times CO_2 \times$ PFD interaction ($P \leq 0.05$) in Table 1.

At the higher PFD levels, net O_2 evolution was greater under elevated CO_2 than ambient CO_2 across both O_2 levels ($CO_2 \times$ PFD; $P = 0.05$), although the difference was more pronounced at 21% O_2 than 2% O_2 (Fig. 1c & d). The greater net O_2 evolution under elevated CO_2 versus ambient CO_2 at 21% O_2 in part reflects the greater rate of net CO_2 assimilation under elevated CO_2.

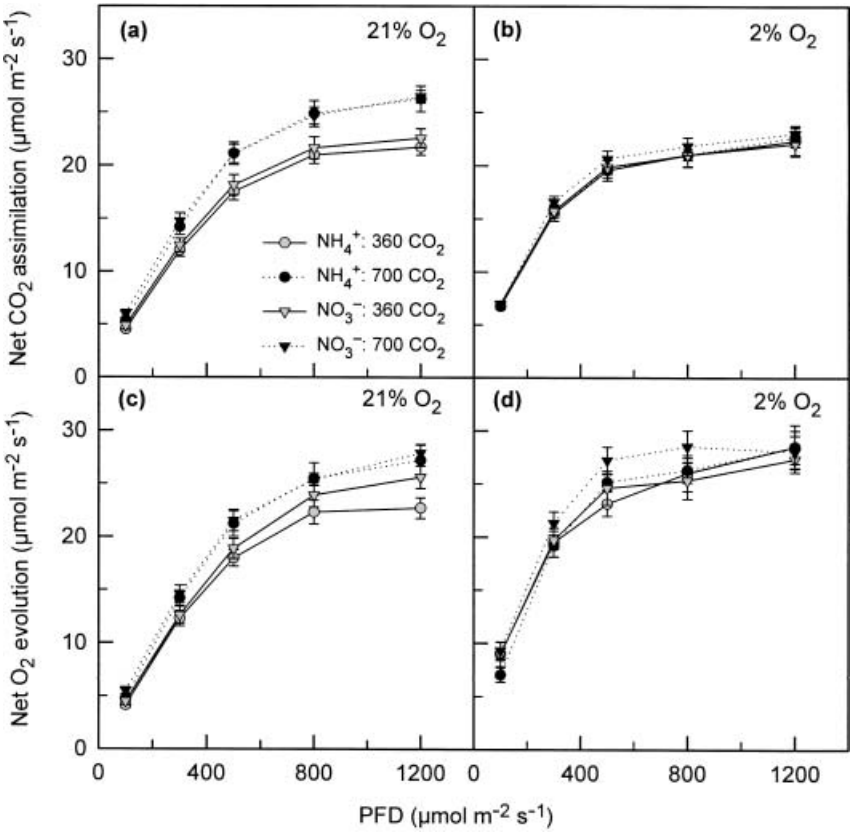

Figure 1. Net CO_2 assimilation and net O_2 evolution in tomato leaves as a function of photosynthetic photon flux density (PFD). Plants were grown in controlled environmental chambers under ambient CO_2 (360 μmol mol^{-1}) and O_2 (21%) conditions, and then measured at 360 (light symbols) or 700 (dark symbols) μmol mol^{-1} CO_2 and under 2% (panels b & d) or 21% (panels a & c) O_2. Either NH_4^+ (circles) or NO_3^- (triangles) was the sole nitrogen source during measurements. The means are shown ±SE for six to nine replicates per treatment. Some symbols are hidden by those of other treatments.

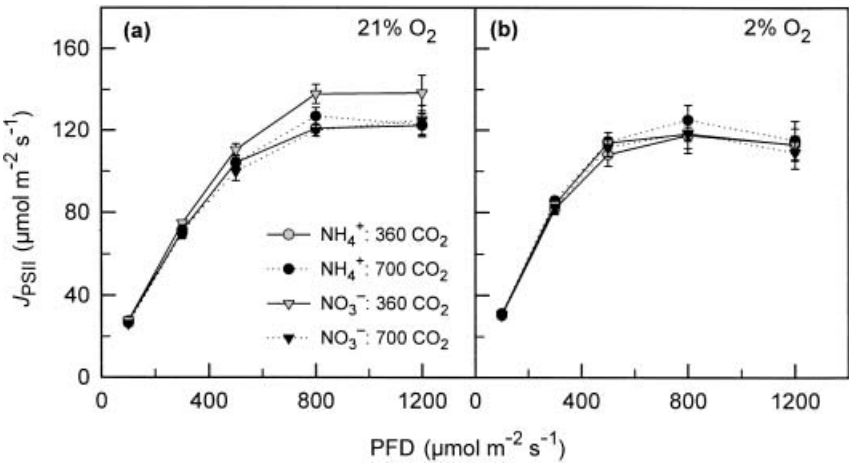

Figure 2. Estimated rate of linear electron transport through photosystem II (J_{PSII}) in tomato leaves as a function of photosynthetic photon flux density (PFD). Plant growth and measurements conditions are the same as Fig. 1. The means are shown ±SE for five to six replicates per treatment. Some symbols are hidden by those of other treatments.

Net O_2 evolution and J_{PSII} estimated from chlorophyll fluorescence were both used to assess electron transport rate. Nitrogen form by itself (i.e. at all PFD levels and under all CO_2 and O_2 treatments) did not have a statistically significant effect on either net O_2 evolution or J_{PSII} (Table 1). Net O_2 evolution at the highest PFD level under ambient CO_2 and 21% O_2, however, was slightly higher (11%) for the NO_3^- than the NH_4^+ treatment (Fig. 1c). Similarly, J_{PSII} at the two highest light levels under ambient CO_2 and 21% O_2 was slightly higher (11–13%) for the NO_3^- than the NH_4^+ treatment (Fig. 2a). Under ambient CO_2 and 2% O_2, neither net O_2 evolution (Fig. 1d) nor J_{PSII} (Fig. 2b) differed significantly between the N treatments.

The assimilatory quotients of gas exchange (AQ_G) were calculated at each PFD level using simultaneous measurements of CO_2 and O_2 exchange to assess NO_3^- assimilation. The AQ_G is the ratio of net CO_2 assimilation to net O_2 evolution, and differences in AQ_G under the two nitrogen sources should reflect the amount of NO_3^- assimilation. Under high PFD and ambient atmospheric conditions (21% O_2, 360 µmol mol^{-1} CO_2), the AQ_G was lower in the NO_3^- than the NH_4^+ treatment (Fig. 3a). No response to the

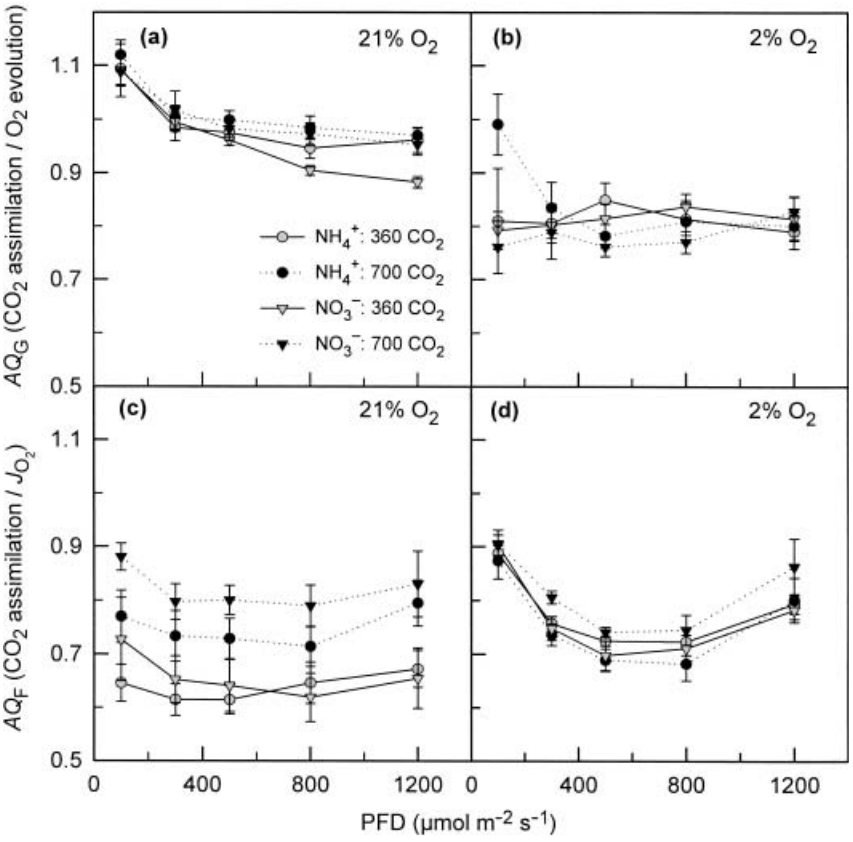

Figure 3. The assimilatory quotient (AQ) in tomato leaves as a function of photosynthetic photon flux density (PFD). The AQ is calculated as the ratio of net CO_2 assimilation to either net O_2 evolution from gas exchange (AQ_G) or estimated gross O_2 evolution (J_{O2}) from chlorophyll fluorescence (AQ_F). Plant growth and measurement conditions are the same as Fig. 1. The means are shown ±SE for five to nine replicates per treatment. Some symbols are hidden by those of other treatments.

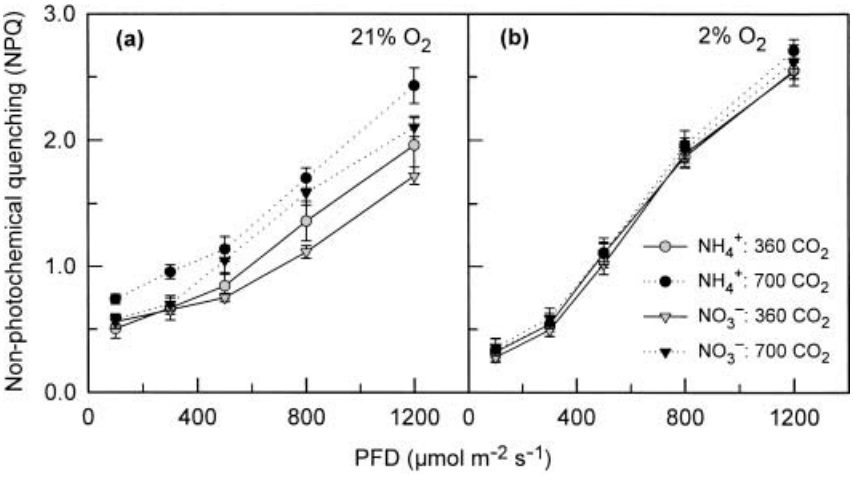

Figure 4. Non-photochemical quenching (NPQ) of chlorophyll fluorescence as a function of photosynthetic photon flux density (PFD). Plant growth and measurement conditions are the same as Fig. 1. The means are shown ±SE for five to nine replicates per treatment. Some symbols are hidden by those of other treatments.

N treatments was apparent under elevated CO_2 at 21% O_2. This indicates greater NO_3^- assimilation under ambient CO_2 than under elevated CO_2 at high PFD. Under 2% O_2, the AQ_G was not affected by N form (Fig. 3b). These results are highlighted in Table 1 by the $N \times O_2 \times CO_2$ ($P \leq 0.05$) and the $N \times CO_2 \times PFD$ ($P \leq 0.01$) interactions. In addition to these interactions, the AQ_G was significantly greater under 21% O_2 than 2% O_2 ($P \leq 0.01$).

An assimilatory quotient (AQ_F) was also calculated as the ratio of net CO_2 assimilation to the estimated gross rate of O_2 evolution (J_{O2}) from chlorophyll fluorescence. In contrast to AQ_G, no differences in AQ_F were apparent due to the N treatments (see Discussion). The AQ_F was influenced by CO_2 and O_2 concentration ($CO_2 \times O_2$; $P \leq 0.01$). The AQ_F values at 21% O_2 were consistently lower under ambient CO_2 than under elevated CO_2, whereas the AQ_F values at 2% O_2 were fairly similar to elevated CO_2 at 21% O_2 (Figs 3c & d). This indicates a greater number of electrons per CO_2 fixed under ambient CO_2 and 21% O_2 than under diminished (elevated CO_2, 21% O_2) or non-photorespiring conditions (2% O_2).

The results for non-photochemical quenching (NPQ) included a complex four-way $N \times O_2 \times CO_2 \times PFD$ interaction (Table 1, $P \leq 0.05$). The NO_3^- treatment had lower values of NPQ than the NH_4^+ treatment at 21% O_2 with the response varying by the level of CO_2 and PFD (Fig. 4a). Lower NPQ in the NO_3^- treatment was apparent only at high PFD under ambient CO_2, whereas NPQ was lower at all PFD levels under elevated CO_2. No differences in NPQ occurred at 2% O_2. Photochemical quenching (q_P) was not affected by the N treatments (Table 1; data not shown).

DISCUSSION

Photorespiration expends a considerable amount of reductant and ATP from photosynthetic electron transport to re-assimilate NH_4^+ and to refix CO_2 (Leegood *et al.* 1995; Wingler *et al.* 2000). Similarly, photo-assimilation of NO_3^- to NH_4^+ oxidizes NAD(P)H and reduced ferredoxin. Thus, if a leaf has limited amounts of these reductants, one might expect the highest rates of NO_3^- photo-assimilation to occur under high light and non-photorespiring conditions. As expected, we observed NO_3^- photo-assimilation in tomato at high, but not at low PFD, based on differences in AQ_G between NO_3^- and NH_4^+ treatments (ΔAQ). However, NO_3^- assimilation was apparent only under ambient CO_2 (360 μmol mol^{-1}) and O_2 (21%) conditions, which promote photorespiration, and not under conditions where photorespiration was diminished (700 μmol mol^{-1} CO_2, 21% O_2) or negligible (2% O_2). These results are consistent with those of our short-term gas exchange experiments with wheat in which elevated CO_2 concentrations inhibited the photo-assimilation of NO_3^- at 21% O_2 (Bloom *et al.* 2002).

Under ambient CO_2 and O_2 conditions, the net O_2 evolution due to NO_3^- assimilation at the highest PFD level was about 10% based on the AQ values from gas exchange (AQ_G). Nitrate assimilation was not clearly reflected in the AQ values calculated from net CO_2 assimilation and gross O_2 evolution from chlorophyll fluorescence (AQ_F) (Table 1). Whereas net CO_2 and O_2 exchange were measured in parallel over the same leaf area, chlorophyll fluorescence was measured in the centre of only one of the three tomato leaflets. This and other factors such as slight differences in irradiance over the surface of the leaflets may complicate comparisons of chlorophyll fluorescence measured on a small area of the leaf with the whole-leaf gas exchange measurements (Haupt-Herting & Fock 2000; Ruuska *et al.* 2000). Simultaneous measurements of gas exchange and chlorophyll fluorescence in our laboratory on maize leaves (*Zea mays*) show better agreement between AQ_G and AQ_F (Asaph Cousins & Arnold Bloom, unpublished results). It is unlikely that estimates of NO_3^- assimilation were biased due to the use of net O_2 exchange rather than gross O_2 evolution through photosystem II since both measures of O_2 evolution were 11–13% higher under the NO_3^- than the NH_4^+ treatment under ambient CO_2 and O_2 concentrations.

Values of AQ from gas exchange measurements of leaves and entire shoots (AQ_G) commonly range between 0.8 and 1.2 (e.g. Kaplan & Bjorkman 1980; Cen *et al.* 2001; Smart

& Bloom 2001; Bloom *et al*. 2002). Theoretically, AQ_G can reach as high as 1.33 if a large proportion of the carbon assimilated enters into organic acids, free amino acids, or nucleic acids, whereas AQ_G drops below 1.0 if the shoots are assimilating NO_3^- or if a large proportion of the carbon assimilated enters protein, lipids, or lignin (Kaplan & Bjorkman 1980; Cen *et al*. 2001). Our AQ_G values for tomato were 0.88–1.12 under 21% O_2 and slightly lower, 0.76–0.99, under 2% O_2. Previous studies have also reported that AQ_G declines under 2% O_2 (Fock, Hilgenberg & Egle 1972; Kaplan & Bjorkman 1980).

For many of our treatments, AQ_G and AQ_F declined as PFD increased from 100 to 300 μmol m^{-2} s^{-1} (Fig. 3). This might reflect a shift in the balance between respiratory O_2 consumption and CO_2 production as light levels increased from just above the compensation point (Hoefnagel, Atkin & Wiskich 1998). Another possibility is that plants might allocate a greater proportion of their carbon to nucleic or amino acids when operating near the light compensation point and might generate relatively more lignin or lipids under increasing light levels. Clearly, changes in *AQ* that are independent of nitrogen source deserve further examination.

In C_3 plants, a doubling of CO_2 concentration typically increases carbon fixation by 25% or more in short-term studies under 21% O_2 (Stitt 1991; Curtis 1996). Net CO_2 assimilation in our study increased by about 20% at the higher PFD levels when CO_2 was doubled under 21% O_2 (Fig. 1a). No stimulation of net CO_2 assimilation occurred under 2% O_2 at high PFD (Fig. 1b) possibly due to a limitation on the rate of photosynthesis by end product formation, primarily starch and sucrose synthesis. End product limitation appears to be common in tomato (Sage & Sharkey 1987; Micallef *et al*. 1995) and occurs under low O_2, high CO_2, and high PFD conditions that favour high rates of triose phosphate production by the chloroplast (Häusler, Schlieben & Flügge 2000).

There are several factors that might help to explain why NO_3^- assimilation was only apparent under ambient CO_2 and O_2 conditions:

1 The re-assimilation of NH_3 produced by the photorespiratory nitrogen cycle is essential for maintaining nitrogen status (for a review, see Wingler *et al*. 2000). If the recycling of NH_3 is inefficient and leaf emissions of NH_3 are high as a consequence, then an acceleration of primary NO_3^- assimilation would be needed to balance or even overcompensate for this loss. Leaf NH_3 emissions, however, are considered to be negligible relative to photorespiratory NH_3 production (Mattsson *et al*. 1997; Schjoerring *et al*. 2000). Thus, it is unlikely that an acceleration of NO_3^- assimilation occurred to account for NH_3 loss.

2 A lowering of photorespiratory capacity in barley mutants deficient in glycine decarboxylase leads to an enhanced reduction state and over-energization of chloroplasts (Igamberdiev *et al*. 2001). In contrast, photorespiration in wild-type plants serves as an important redox transfer mechanism that increases the cytosolic NADH/NAD ratio via the export of malate from the chloroplast as described by Backhausen, Kitzmann & Scheibe (1994). Because the first step of NO_3^- assimilation (i.e. the reduction of NO_3^- to NO_2^-) occurs in the cytosol and uses NADH from the malate shuttle, this may explain why we observed NO_3^- assimilation to be greater when photorespiration was highest, that is, under ambient CO_2 and 21% O_2.

3 In addition to the malate shuttle, reductant for the NO_3^- to NO_2^- reaction can be provided by the conversion of triose phosphate to organic acids in the cytosol (Noctor & Foyer 1998). Triose phosphate is also an intermediate in the formation of sucrose and starch. Given the apparent end product limitation in our study on the rate of photosynthesis by starch and sugar formation under diminished photorespiratory conditions, one might expect that more triose phosphate would have been diverted from sucrose production to the production of organic acids and NADH for NO_3^- assimilation under 2% O_2. However, this did not seem to occur based on the lack of apparent NO_3^- assimilation under 2% O_2 in our study.

4 Nitrate photo-assimilation may be a means of photoprotection from high irradiance (Zhu *et al*. 2000). In our study, non-photochemical quenching (NPQ) at high irradiance was indeed lower when plants received NO_3^- rather than NH_4^+ under ambient CO_2 and O_2 conditions. However, NPQ was also lower at elevated CO_2 and 21% O_2.

Of these possibilities, increased cytosolic NADH due to photorespiration (hypothesis B) appears to best explain the higher rates of NO_3^- assimilation under ambient CO_2 and O_2 conditions. As indicated above, inefficiencies in photorespiratory NH_3 recycling, conversion of triose phosphate to organic acids and NADH, and nitrate assimilation as a means of photoprotection seem unlikely to explain the results based on previous studies or the results presented in this study.

In addition to tomato, NO_3^- assimilation has previously been shown to decrease under elevated CO_2 in wheat (Bloom *et al*. 2002). Gas exchange measurements, NO_3^- reductase activity, shoot protein, and shoot biomass of wheat plants grown under either ambient or elevated CO_2 and either NH_4^+ or NO_3^- as a nitrogen source all indicated an inhibition of NO_3^- assimilation under elevated CO_2. In another recent study on wheat, Novitskaya *et al*. (2002) found no consistent trends in leaf amino acid levels with short-term exposure to various CO_2 concentrations, but suggested that NO_3^- assimilation might increase under negligible photorespiration (2% O_2) based on trends in leaf malate levels. Surprisingly, net CO_2 assimilation in this second study did not increase under elevated CO_2 even though the plants were grown at ambient CO_2 levels. Distinct approaches as well as differences in conditions, such as plant age and nutrient levels that can influence NO_3^- assimilation (Geiger *et al*. 1998; Geiger *et al*. 1999), may help to

explain some of the discrepancies among these studies on wheat.

In conclusion, conditions that diminish photorespiration, either elevated CO_2 or low O_2 limited leaf NO_3^- photoassimilation in short-term experiments on tomato. Consequently, complex interactions between photorespiratory metabolism and NO_3^- assimilation may be more important than previously recognized in plant leaves.

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