Photosynthetic induction upon transfer from low to high light is affected by leaf nitrogen content in tomato

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Running head:

Photosynthesis under fluctuating light in tomato

Abstract

The response of photosynthetic CO_2 assimilation to changes of illumination affects plant growth and crop productivity under natural fluctuating light conditions. However, the effects of nitrogen (N) supply on photosynthetic physiology after transition from low to high light are seldom studied. To elucidate this, we measured gas exchange and chlorophyll fluorescence under fluctuating light in tomato (*Solanum lycopersicum*) seedlings grown with different N conditions. After transition from low to high light, the induction speeds of net CO_2 assimilation (A_N), stomatal conductance (g_s) and mesophyll conductance (g_m) delayed with the decline in leaf N content. The times to reach 90% of maximum A_N, g_s and g_m were negatively correlated to leaf N content. This delayed photosynthetic induction in plants grown under low N concentration was mainly caused by the slow induction response of g_m rather than that of g_s. Furthermore, the photosynthetic induction upon transfer from low to high light was hardly limited by photosynthetic electron flow. These results indicate that decreased leaf N content declines carbon gain under fluctuating light in tomato. Increasing the induction kinetics of g_m has the potential to enhance the carbon gain of field crops grown in infertile soil.

Keywords: fluctuating light, nitrogen, photosynthesis, mesophyll conductance, photosynthetic limitation.

Introduction

Plants capture light energy to produce chemical energy ATP and NADPH, which are used to drive nitrogen assimilation and the conversion of CO_2 to sugar. Enhancing net CO_2 assimilation rate (A_N) is thought to be one of the most important targets for improving plant growth and crop productivity (Yamori *et al.* 2016a; Kromdijk *et al.* 2016; South, Cavanagh, Liu & Ort 2019). Many previous studies indicated that increasing A_N under constant high light can boost plant biomass (Kebeish *et al.* 2007; Timm *et al.* 2012, 2015). Recently, some studies reported that the response of A_N to increases of illumination significantly affects the carbon gain and thus influences plant growth (Slattery, Walker, Weber & Ort 2018; Adachi *et al.* 2019; Kimura, Hashimoto-Sugimoto, Iba, Terashima & Yamori 2020; Yamori, Kusumi, Iba & Terashima 2020; Zhang, Kaiser, Marcelis, Yang & Li 2020). Therefore, altering the photosynthetic performance under dynamic illumination is a promising way to improving photosynthesis under natural fluctuating light (FL) conditions.

Plants grown under high nitrogen (N) concentration usually have higher biomass than plants grown under low N concertation (Makino 2011). An important explanation for this is that leaf photosynthetic capacity is related to the leaf N content in many higher plants (Yamori, Nagai & Makino 2011; Li *et al.* 2020), since stromal enzymes and thylakoid proteins account for the majority of leaf N (Makino & Osmond 1991; Sudo, Makino & Mae 2003; Takashima, Hikosaka & Hirose 2004). Furthermore, stomatal conductance (g_s) and mesophyll conductance (g_m) under constant high light are also increased in plants grown under high N concentration, which speeds up CO_2 diffusion from atmosphere to chloroplast carboxylation sites and thus favors the operation of A_N under constant high light (Yamori *et al.* 2011). However, little is known about the effects of leaf N content on non-steady-state photosynthetic performances under FL.

Under natural field conditions, light intensity exposed on leaf surface dynamically changes on timescales from milliseconds to hours (Pearcy 1990; Slattery *et al.* 2018). Furthermore, FL and N deficiency usually occurs concomitantly, but how FL and N deficiency interact to influence photosynthetic physiology in crop plants is poorly understood. After a sudden transitioning from low to high light, the gradual increase of A_N is termed

"photosynthetic induction". Recent studies indicated that the induction response of A_N was significantly affected by the induction speed of g_s (De Souza, Wang, Orr, Carmo-Silva & Long 2020; Kimura *et al.* 2020). Gene expression plays a crucial role in the induction response of g_s under FL. For example, the *slow anion channel-associated 1* (*slac1*), *open stomata 1* (*ost1*) and abscisic acid deficient *flacca* mutants, and the *proton ATPase translocation control 1* (*PATROL1*) overexpression line had faster stomatal opening responses than WT-types in *Arabidopsis thaliana*, rice and tomato (Kaiser, Morales, Harbinson, Heuvelink & Marcelis 2020; De Souza *et al.* 2020; Kimura *et al.* 2020; Yamori *et al.* 2020). Furthermore, the stomatal opening during photosynthetic induction can be affected by environment conditions such as target light intensity, magnitude of change, g_s at low light, the time of day and vapor pressure deficit (Kaiser *et al.* 2020; Sakoda *et al.* 2020; Eyland, van Wesemael, Lawson & Carpentier 2021). However, there have been few studies that examined the effect of leaf N content on the induction response of g_s after transition from low to high light (Li *et al.* 2020).

In addition to g_s, g_m is a major factor affecting CO_2 concentration in chloroplast, because g_m determines the CO_2 diffusion from intercellular space into the chloroplast (Flexas *et al.* 2013; Carriquí *et al.* 2015). In general, g_m can be determined by structure across leaf profiles, genetic types, biochemical components and environmental conditions (Yamori *et al.* 2011; Xiong *et al.* 2015; Théroux-Rancourt & Gilbert 2017). Previous studies have highlighted that g_m is the most important limiting factor for A_N in many angiosperms (Peguero-Pina *et al.* 2017; Xiong, Douthe & Flexas 2018; Yang, Huang, Yang, Chang & Zhang 2018b). Short-term response of g_m to light intensity has been determined and found that it varies between plant species (Tazoe, Von Caemmerer, Badger & Evans 2009; Yamori, Evans & Von Caemmerer 2010a; Xiong *et al.* 2018; Yang, Hu & Huang 2020). However, the induction response of g_m after transition from low to high light is little known. The g_m level under constant light is also significantly affected by leaf N content (Yamori *et al.* 2011). Furthermore, the rapid responses of g_m to CO_2 concentration and temperature were also affected by leaf N content (Xiong *et al.* 2015). However, no studies have elucidated the effect of leaf N content on induction response of g_m upon transfer from low to high light.

In this study, we aimed to characterize the effects of leaf N content on induction kinetics of A_N, g_s and g_m after a sudden transition from low to high light. Gas exchange and chlorophyll fluorescence were measured in tomato plants grown under contrasting N concentrations. The dynamic limitations of g_s, g_m and biochemical factors imposed on A_N were analyzed based on the biochemical model for C3 photosynthesis (Farquhar, von Caemmerer & Berry 1980). The effects of leaf N content on photosynthetic performances during photosynthetic induction were revealed.

Materials and methods

Plant materials and growth conditions

Tomato (*Solanum lycopersicum* cv. Hupishizi) plants were grown in a greenhouse with the light condition of 40% full sunlight. The day/night air temperatures were approximately 30/20ºC, the relative air humidity was approximately 60%-70%, and the maximum light intensity exposed to leaves was approximately 800 μmol photons m^{-2} s^{-1}. Plants were grown in 19-cm plastic pots with humus soil and the initial soil N content was 2.1 mg/g. Plants were fertilized with Peters Professional's water solution (N:P:K = 15:4.8:24.1) or water as follows: high nitrogen (HN, 0.15 g N/plant every two days), middle nitrogen (MN, 0.05 g N/plant once a week) and low nitrogen (LN, 0 mM N/plant). 0.3% water solution were used for fertilization, and the nitrogen sources were 24% $(NH_4)_3PO_4$, 65% KNO_3 and 9.5% CH_4N_2O. To prevent any water stress, these plants were watered every day. After cultivation for one month, youngest fully developed leaves were used for measurements.

Gas exchange and chlorophyll fluorescence measurements

An open gas exchange system (LI-6400XT; Li-Cor Biosciences, Lincoln, NE, USA) was used to simultaneously measure gas exchange and chlorophyll fluorescence. Measurements were performed at a leaf temperature of approximately 25ºC, leaf-to-air vapour pressure deficit of 1.2-1.4 kpa, and flow rate of air through the system of 300 μmol/s. To measure photosynthetic induction after a short-term shadefleck, leaves were firstly adapted to a light intensity of 1500 μmol photons m^{-2} s^{-1} and air CO_2 concentration of 400 μmol mol^{-1} for >20 min until A_N and

g_s reached steady-state. Then, leaves were subjected to 5 min of low light (100 μmol photons m^{-2} s^{-1}) followed by 30 min of high light (1500 μmol photons m^{-2} s^{-1}), and gas exchange and chlorophyll fluorescence were logged every minute. iWUE was calculated as iWUE = A_N/g_s. The relative A_N, g_s and g_m curves were obtained from the standardization against the maximum values after 30 min photosynthetic induction at high light. The time required to reach 90% of the maximum A_N, g_s and g_m was estimated by the first time at which the relative values were higher than 90%. After photosynthetic induction measurement, the response of CO_2 assimilation rate to incident intercellular CO_2 concentration (A/C_i) curves were measured by decreasing the CO_2 concentration to a lower limit of 50 μmol mol^{-1} and then increasing stepwise to an upper limit of 1500 μmol mol^{-1}. For each CO_2 concentration, photosynthetic measurement was completed in 3 min. Using the A/C_i curves, the maximum rates of RuBP regeneration (J_{max}) and carboxylation (V_{cmax}) were calculated (Long & Bernacchi 2003).

The quantum yield of PSII photochemistry was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty, Briantais & Baker 1989), where F_m' and F_s represent the maximum and steady-state fluorescence after light adaptation, respectively (Baker 2004). The total electron transport rate through PSII (J_{PSII}) was calculated as follows (Krall & Edwards 1992):

$$J_{PSII} = \Phi_{PSII} \times PPFD \times L_{abs} \times 0.5$$

where PPFD is the photosynthetic photon flux density and leaf absorbance (L_{abs}) is assumed to be 0.84. We applied the constant of 0.5 based on the assumption that photons were equally distributed between PSI and PSII.

Estimation of mesophyll conductance and chloroplast CO_2 concentration

Mesophyll conductance was calculated according to the following equation (Harley, Loreto, Di Marco & Sharkey 1992):

$$g_m = \frac{A_N}{C_i - \Gamma^*(J_{PSII} + 8(A_N + R_d))/(J_{PSII} - 4(A_N + R_d))}$$

where A_N represents the net rate of CO_2 assimilation; C_i is the intercellular CO_2 concentration; Γ^* is the CO_2 compensation point in the absence of daytime respiration (Yamori, Noguchi, Hikosaka & Terashima 2010b; von Caemmerer & Evans 2015), and we used a typical value of 40 μmol mol^{-1} in our current study (Xiong *et al.* 2018). Respiration rate in the dark (R_d)

was considered to be half of the dark-adapted mitochondrial respiration rate as measured after 10 min of dark adaptation (Carriquí *et al.* 2015).

Based on the estimated g_m, we then calculated the chloroplast CO_2 concentration (C_c) according to the following equation (Long & Bernacchi 2003; Warren & Dreyer 2006):

$$C_c = C_i - \frac{A_N}{g_m}$$

Quantitative limitation analysis of A_N

Relative photosynthetic limitations were assessed as follows (Grassi & Magnani 2005):

$$L_s = \frac{g_{tot}/g_s \times \partial A_N / \partial C_c}{g_{tot} + \partial A_N / \partial C_c}$$

$$L_{mc} = \frac{g_{tot}/g_m \times \partial A_N / \partial C_c}{g_{tot} + \partial A_N / \partial C_c}$$

$$L_b = \frac{g_{tot}}{g_{tot} + \partial A_N / \partial C_c}$$

where L_s, L_{mc}, and L_b represent the relative limitations of stomatal conductance, mesophyll conductance, and biochemical capacity, respectively, in setting the observed value of A_N. g_{tot} is the total conductance of CO_2 between the leaf surface and sites of RuBP carboxylation (calculated as $1/g_{tot} = 1/g_s + 1/g_m$).

SPAD index and nitrogen content measurements

A handy chlorophyll meter (SPAD-502 Plus; Minolta, Tokyo, Japan) was used to non-destructively measure the SPAD index (relative content of chlorophyll per unit leaf area) of leaves used for photosynthetic measurements. Thereafter, leaf area was measured using a LI-3000A portable leaf area meter (Li-Cor, Lincoln, NE, USA). After leaf material was dried at 80°C for 48 hours, dry weight was measured and leaf N content was determined with a Vario MICRO Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) (Sakowska *et al.* 2018).

Results

Effect of leaf N content on steady-state physiological characteristics under high light

The leaf N content in LN-, MN- and HN-plants were 0.42 ± 0.03, 0.71 ± 0.3 and 1.2 ± 0.07 g

178 m^{-2}, respectively (Table 1). The HN-plants displayed the highest relative chlorophyll content,
179 measured by SPAD value, followed by MN- and LN-plants. After 30 min light adaptation at
180 1500 μmol photons m^{-2} s^{-1} and 400 μmol mol^{-1} CO_2 concentration, HN-plants had the highest
181 net CO_2 assimilation rate (A_N), stomatal conductance (g_s), mesophyll conductance (g_m) and
182 electron transport rate (ETR). Therefore, the steady-state photosynthetic capacities were
183 significantly affected by leaf N content. Furthermore, HN-, MN- and LN-plants showed slight
184 difference in g_s but significant difference in g_m, indicating that g_m is more responsive to leaf N
185 content than g_s in tomato.
186
187 **Effects of leaf N content on photosynthetic induction upon transfer from low to high**
188 **light**
189 During this photosynthetic induction after 5 min of shadefleck, HN-plants showed the highest
190 induction speeds of A_N, g_s and g_m, followed by MN- and LN-plants (Fig. 1). The time required
191 to reach 90% of the maximum A_N (t_{90AN}) significantly increased with the decrease in leaf N
192 content (Fig. 1G). The time required to reach 90% of the maximum g_s and g_m (t_{90gs} and t_{90gm},
193 respectively) were significantly shorter in HN-plants than MN- and LN-plants, whereas t_{90gs}
194 and t_{90gm} did not differ significantly between MN- and LN-plants (Fig. 1G). Interestingly, t_{90gm}
195 was lower than t_{90gs} in all plants. The higher t_{90gs} and t_{90AN} in MN- and LN-plants was partially
196 related to the relatively lower initial g_s prior to light change (Fig. S1). Within the first 15
197 minutes after transition from low to high light, all plants showed similar intrinsic water use
198 efficiency (iWUE) (Fig. S2). However, during prolonged photosynthetic induction, HN-plants
199 displayed much higher iWUE than MN- and LN-plants (Fig. S2). Further analysis found that
200 leaf N content was negatively correlated to t_{90AN}, t_{90gs} and t_{90gm} (Fig. 2). Therefore, leaf N
201 content plays a crucial role in affecting the induction responses of A_N, g_s and g_m after
202 transition from low to high light. The comparative extent of the reductions of t_{90AN} was more
203 correlated to t_{90gm} than t_{90gs} (Fig. 3A). Furthermore, the change in A_N during photosynthetic
204 induction was more related to g_m than g_s (Fig. 3B&C). These results suggest that, upon
205 transfer from low to high light, g_m plays a more important role in determining the induction
206 response of A_N than g_s.

Effects of leaf N content on intercellular and chloroplast CO_2 concentrations upon transfer from low to high light

We calculated the response kinetics of intercellular (C_i) and chloroplast CO_2 concentration (C_c) using A_N, g_s and g_m. After transitioning from low to high light, C_i and C_c gradually increased in all plants (Fig. 4). HN-plants had the lowest values of C_i and C_c after photosynthetic sufficient photosynthetic induction. The change in A_N during photosynthetic induction was tightly and positively correlated to C_c in all plants, suggesting the importance of C_c in determining A_N. Because C_c can be affected by g_s and g_m, we analyzed the relationships between C_c, g_s and g_m. Compared with g_s, a smaller change in g_m could result in a larger change in C_c (Fig. 5), suggesting that the change of C_c upon transfer from low to high light was more determined by g_m than g_s.

Effects of leaf N content on relative limitations of photosynthesis upon transfer from low to high light

After transition from low to high light, the limitations of photosynthesis by g_s (L_{gs}), g_m (L_{gm}) and biochemical factors (L_b) changed slightly in HN-plants (Fig. 6). In MN- and LN-plants, L_{gs} gradually decreased over time. Within the first 15 min, L_{gs} was lower in HN-plants than MN- and LN-plants. However, the LN-plants had the lowest L_{gs} after sufficient photosynthetic induction. L_{gm} was also maintained stable during whole photosynthetic induction in MN- and LN-plants, but L_b gradually increased from 0.3 to 0.5 in them. Therefore, leaf N content could affect the kinetics of relative limitations of photosynthesis during photosynthetic induction after transfer from low to high light. To explore whether the induction of A_N is limited by photosynthetic electron transport, we estimated the dynamic change of electron transport rate (ETR). Upon a sudden increase in illumination, ETR rapidly increased and the ETR/(A_N + R_d) ratio first increased and then gradually decreased in all plants (Fig. 7). These results indicated that the activation speed of ETR was much faster than that of A_N. Therefore, during photosynthetic induction the limitation of ETR imposed to A_N was negligible in all samples.

Discussion

Leaf N content plays an important role in determining photosynthesis, plant growth and crop productivity (Makino 2011). Under natural field conditions, FL and N deficiency usually occurs concomitantly. However, it is unknown how FL and N deficiency interact to influence photosynthetic physiology in crop plants. In this study, we here for the first time examined the effects of leaf N content on photosynthetic induction after transition from low to high light in tomato. We found that leaf N content significantly affected the induction responses of g_s and g_m and thus affected induction kinetics of A_N. However, the activation speed of photosynthetic electron flow was not influenced by leaf N content. Therefore, the effect of leaf N content on photosynthetic induction was more attributed to the induction kinetics of diffusional conductance rather than the activation speed of electron transport.

In addition to steady-state photosynthetic capacity under high light, the photosynthetic responses to the changes in illumination significantly affect the carbon gain and plant biomass (Adachi *et al.* 2019; Kimura *et al.* 2020; Zhang *et al.* 2020). Many previous studies have documented that leaf N content influences the steady-state photosynthetic performances under high light (Evans & Terashima 1988; Makino & Osmond 1991), but little is known about the influence of leaf N content on photosynthetic induction under FL conditions. Similar to previous studies, the maximum steady-state A_N under high light significantly declined with the decrease in leaf N content (Table 1). Moreover, we here found that, after transition from low to high light, the HN-plants showed much faster induction response of A_N than MN- and LN-plants (Fig. 1). The time required to reach 90% of the steady-state of photosynthesis (t_{90AN}) was negatively correlated to leaf N content (Fig. 2). Therefore, leaf N content significantly affect the photosynthetic induction after transition from low to high light in tomato. This finding is similar to the photosynthetic induction of dark-adapted leaves among canola genotypes (*Brassica napus* L.) (Liu, Zhang, Estavillo, Luo & Hu 2021), but was inconsistent with the phenomenon in soybean (Li *et al.* 2020). In soybean, the induction rate of A_N under high light after shading for 5 min was very fast (Pearcy, Krall & Sassenrath-Cole 1996; Li *et al.* 2020). Furthermore, this fast photosynthetic induction in soybean was not affected by leaf N content (Li *et al.* 2020). Therefore, the effect of leaf N content on fast photosynthetic

induction following shadefleck depends on the species and on growth conditions. In MN- and LN-plants of tomato, the delayed induction of A_N caused a larger loss of carbon gain under FL. This finding provides insight into why plants grown under low N concentrations display reduction of plant biomass under natural field FL conditions.

After transition from low to high light, the time to reach the maximum C_c was less in HN-plants than MN- and LN-plants (Fig. 4). Furthermore, tight and positive relationships were found between C_c and A_N in all plants (Fig. 4). These results suggested that the induction response of A_N was largely determined by the change of CO_2 concentration in the site of RuBP carboxylation. The value of C_c in a given leaf is largely affected by CO_2 diffusional conductance, including g_s and g_m (Sagardoy *et al.* 2010; Carriquí *et al.* 2015; Yang *et al.* 2018b). However, it is unclear whether the photosynthetic induction of A_N upon transfer from low to high light is more determined by the induction response of g_s or g_m. We found that the induction responses of g_s and g_m were largely delayed in MN- and LN-plants than HN-plants (Fig. 1), and the induction rates of g_s and g_m were negatively correlated to leaf N content (Fig. 2). Furthermore, the change of C_c during photosynthetic induction was more related to g_m rather than g_s (Fig. 5), pointing out the important role of g_m response in determining C_c upon transfer from low to high light. Therefore, the delayed photosynthetic induction of A_N in plants grown under low N concentrations was more attributed to the slower induction response of g_m than g_s.

In HN-plants of tomato, photosynthetic limitations by g_s, g_m and biochemical factors changed slightly upon transfer from low to high light. Meanwhile, g_s imposed to the smallest limitation to A_N, owing to the high levels of g_s (Fig. 6). Therefore, improving the induction response of g_s might have a minor factor for improving photosynthesis under FL in HN-plants of tomato under optimal conditions (Kaiser *et al.* 2020). By comparison, increased g_s has a significant effect on photosynthetic CO_2 assimilation under FL in *Arabidopsis thaliana* and rice (Kimura *et al.* 2020; Yamori *et al.* 2020). These results indicate that the effects of altered g_s kinetics on photosynthesis under FL is species dependent. In MN- and LN-plants, the relatively slower kinetics of g_s led to a higher L_{gs} of A_N during the initial 15 min after transition from low to high light (Fig. 6). Therefore, altered g_s kinetics would have more

294 significant effects on photosynthetic carbon gain in crop plants grown under low N
295 concentrations.

296 Many previous studies have indicated that g_m act as a major limitation for steady-state A_N
297 under high light in many angiosperms (Peguero-Pina *et al.* 2017; Théroux-Rancourt & Gilbert
298 2017; Yang, Tong, Yu, Zhang & Huang 2018a; Huang, Yang, Wang & Hu 2019). Increasing
299 g_m has been thought to be a potential target for improving crop productivity and water use
300 efficiency under constant high light (Flexas *et al.* 2013; Gago *et al.* 2016). However, the
301 limitation of g_m imposed to A_N under FL is poorly understood. Upon transition from dark to
302 light, the induction response of g_m was much faster than that of g_s, leading to the smallest
303 limitation of g_m imposed to A_N in *Arabidopsis thaliana* and tobacco (Sakoda, Yamori,
304 Groszmann & Evans 2021). Consequently, one concluded that altering g_m kinetics would have
305 little impact on A_N under FL. However, we found that, after transfer from low to high light,
306 L_{gm} was higher than L_{gs} in tomato plants (Fig. 6). Furthermore, the time to reach 90% of A_N
307 was closer to that of g_m rather than that of g_s (Fig. 3). Therefore, altering g_m kinetics would
308 significantly influence A_N upon transfer from low to high light, at least in tomato. These
309 results suggested that the photosynthetic limitation upon transfer from low to high light was
310 largely different from the photosynthetic induction during illumination of dark-adapted leaves.
311 Improving the induction rate of g_m has a potential to enhance carbon gain and plant biomass
312 under natural FL conditions.

313 A recent study reported that, if RuBP regeneration limitation was assumed, electron
314 transport imposed the greatest limitation to A_N during illumination of dark-adapted leaves
315 (Sakoda *et al.* 2021). Based on this result, it is hypothesized that increased activation of
316 electron transport has the potential to enhance carbon gain under naturally FL environments.
317 Controversially, our present study indicated that electron transport was rapidly activated upon
318 transfer from low to high light. After transition from low to high light, the ETR/(A_N + R_d)
319 value rapidly increased to the peak within 1-2 min and then gradually decreased over time
320 (Fig. 7). These results indicated that, upon transfer from low to high light, the induction
321 response of electron transport was much faster than that of A_N, which was consistent to the
322 photosynthetic performance in rice (Yamori, Makino & Shikanai 2016b). Therefore, induction

response of A_N after transition from low to high light was hardly limited by electron transport in tomato. The effect of electron transport on A_N upon transition from low to high light is largely different from that upon transition from dark to light. Therefore, to improve photosynthesis under FL in tomato, more attention should be focused on the induction kinetics of CO_2 diffusional conductance rather than the activation of electron transport.

Conclusions

We studied the effects of leaf N content on photosynthetic induction after transfer from low to high light in tomato. The induction speeds of A_N, g_s and g_m significantly decreased with the decrease in leaf N content. Such delayed photosynthetic induction in plants grown under low N concentration caused a larger loss of carbon gain under FL conditions, which further explained why N deficiency reduced plant biomass under natural FL environments. After transition from low to high light, increasing the induction responses of g_s and g_m has the potential to improve A_N in tomato, especially when plants are grown under low N concentration, whereas photosynthetic induction of A_N was hardly limited by electron transport. Therefore, altering induction kinetics of CO_2 diffusional conductance is likely the most effective target for improving photosynthesis under FL conditions in tomato.

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References

Adachi S., Tanaka Y., Miyagi A., Kashima M., Tezuka A., Toya Y., … Yamori W. (2019) High-yielding rice Takanari has superior photosynthetic response to a commercial rice Koshihikari under fluctuating light. *Journal of Experimental Botany* **70**, 5287–5297.

Baker N.R. (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* **55**, 1607–1621.

352 von Caemmerer S. & Evans J.R. (2015) Temperature responses of mesophyll conductance
353 differ greatly between species. *Plant, Cell and Environment* **38**, 629–637.
354 Carriquí M., Cabrera H.M., Conesa M., Coopman R.E., Douthe C., Gago J., … Flexas J.
355 (2015) Diffusional limitations explain the lower photosynthetic capacity of ferns as
356 compared with angiosperms in a common garden study. *Plant, Cell and Environment* **38**,
357 448–460.
358 Evans J.R. & Terashima I. (1988) Photosynthetic characteristics of spinach leaves grown with
359 different nitrogen treatments. *Plant and Cell Physiology* **29**, 157–165.
360 Eyland D., van Wesemael J., Lawson T. & Carpentier S. (2021) The impact of slow stomatal
361 kinetics on photosynthesis and water use efficiency under fluctuating light. *Plant*
362 *Physiology* **186**, 998–1012.
363 Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of
364 photosynthetic CO2 assimilation in leaves of C3 species. *Planta* **149**, 78–90.
365 Flexas J., Niinemets Ü., Gallé A., Barbour M.M., Centritto M., Diaz-Espejo A., … Medrano
366 H. (2013) Diffusional conductances to CO2 as a target for increasing photosynthesis and
367 photosynthetic water-use efficiency. *Photosynthesis Research* **117**, 45–59.
368 Gago J., Daloso D. de M., Figueroa C.M., Flexas J., Fernie A.R. & Nikoloski Z. (2016)
369 Relationships of Leaf Net Photosynthesis, Stomatal Conductance, and Mesophyll
370 Conductance to Primary Metabolism: A Multispecies Meta-Analysis Approach. *Plant*
371 *Physiology* **171**, 265–279.
372 Genty B., Briantais J.-M. & Baker N.R. (1989) The relationship between the quantum yield of
373 photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica*
374 *et Biophysica Acta (BBA) - General Subjects* **990**, 87–92.
375 Grassi G. & Magnani F. (2005) Stomatal, mesophyll conductance and biochemical limitations
376 to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant,*
377 *Cell and Environment* **28**, 834–849.
378 Harley P.C., Loreto F., Di Marco G. & Sharkey T.D. (1992) Theoretical considerations when
379 estimating the mesophyll conductance to CO2 flux by analysis of the response of
380 photosynthesis to CO2. *Plant Physiology* **98**, 1429–1436.

381 Huang W., Yang Y.-J., Wang J.-H. & Hu H. (2019) Photorespiration is the major alternative
382 electron sink under high light in alpine evergreen sclerophyllous Rhododendron species.
383 *Plant Science*, 110275.
384 Kaiser E., Morales A., Harbinson J., Heuvelink E. & Marcelis L.F.M. (2020) High Stomatal
385 Conductance in the Tomato Flacca Mutant Allows for Faster Photosynthetic Induction.
386 *Frontiers in Plant Science* **11**, 1–12.
387 Kebeish R., Niessen M., Thiruveedhi K., Bari R., Hirsch H.-J., Rosenkranz R., … Peterhänsel
388 C. (2007) Chloroplastic photorespiratory bypass increases photosynthesis and biomass
389 production in Arabidopsis thaliana. *Nature biotechnology* **25**, 593–599.
390 Kimura H., Hashimoto-Sugimoto M., Iba K., Terashima I. & Yamori W. (2020) Improved
391 stomatal opening enhances photosynthetic rate and biomass production in fluctuating
392 light. *Journal of Experimental Botany* **71**, 2339–2350.
393 Krall J.P. & Edwards G.E. (1992) Relationship between photosystem II activity and CO2
394 fixation in leaves. *Physiologia Plantarum* **86**, 180–187.
395 Kromdijk J., Głowacka K., Leonelli L., Gabilly S.T., Iwai M., Niyogi K.K. & Long S.P.
396 (2016) Improving photosynthesis and crop productivity by accelerating recovery from
397 photoprotection. *Science* **354**, 857–861.
398 Li Y.-T., Li Y., Li Y.-N., Liang Y., Sun Q., Li G., … Gao H.-Y. (2020) Dynamic light caused
399 less photosynthetic suppression, rather than more, under nitrogen deficit conditions than
400 under sufficient nitrogen supply conditions in soybean. *BMC Plant Biology* **20**, 339.
401 Liu J., Zhang J., Estavillo G.M., Luo T. & Hu L. (2021) Leaf N content regulates the speed of
402 photosynthetic induction under fluctuating light among canola genotypes (Brassica
403 napus L.). *Physiologia Plantarum* **172**, 1844–1852.
404 Long S.P. & Bernacchi C.J. (2003) Gas exchange measurements, what can they tell us about
405 the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of*
406 *Experimental Botany* **54**, 2393–2401.
407 Makino A. (2011) Photosynthesis, Grain Yield, and Nitrogen Utilization in Rice and Wheat.
408 *Plant Physiology* **155**, 125–129.
409 Makino A. & Osmond B. (1991) Effects of nitrogen nutrition on nitrogen partitioning

between chloroplasts and mitochondria in pea and wheat. *Plant Physiology* **96**, 355–362.

Pearcy R.W. (1990) Sunflecks and photosynthesis in plant canopies. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 421–453.

Pearcy R.W., Krall J.P. & Sassenrath-Cole G.F. (1996) Photosynthesis in Fluctuating Light Environments. In *Photosynthesis and the Environment*. pp. 321–346. Kluwer Academic Publishers, Dordrecht.

Peguero-Pina J.J., Sisó S., Flexas J., Galmés J., García-Nogales A., Niinemets Ü., … Gil-Pelegrín E. (2017) Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytologist* **214**, 585–596.

Sagardoy R., Vázquez S., Florez-Sarasa I.D., Albacete A., Ribas-Carbó M., Flexas J., … Morales F. (2010) Stomatal and mesophyll conductances to CO2 are the main limitations to photosynthesis in sugar beet (Beta vulgaris) plants grown with excess zinc. *New Phytologist* **187**, 145–158.

Sakoda K., Yamori W., Groszmann M. & Evans J.R. (2021) Stomatal , mesophyll conductance , and biochemical limitations to photosynthesis during induction Research Article. 146–160.

Sakoda K., Yamori W., Shimada T., Sugano S.S., Hara-Nishimura I. & Tanaka Y. (2020) Higher stomatal density improves photosynthetic induction and biomass production in Arabidopsis under fluctuating light. *Frontiers in Plant Science* **11**, 1308.

Sakowska K., Alberti G., Genesio L., Peressotti A., Delle Vedove G., Gianelle D., … Miglietta F. (2018) Leaf and canopy photosynthesis of a chlorophyll deficient soybean mutant. *Plant, Cell & Environment* **41**, 1427–1437.

Slattery R.A., Walker B.J., Weber A.P.M. & Ort D.R. (2018) The impacts of fluctuating light on crop performance. *Plant Physiology* **176**, 990–1003.

South P.F., Cavanagh A.P., Liu H.W. & Ort D.R. (2019) Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* **363**, eaat9077.

De Souza A.P., Wang Y., Orr D.J., Carmo□Silva E. & Long S.P. (2020) Photosynthesis across African cassava germplasm is limited by Rubisco and mesophyll conductance at

steady state, but by stomatal conductance in fluctuating light. *New Phytologist* **225**, 2498–2512.

Sudo E., Makino A. & Mae T. (2003) Differences between rice and wheat in ribulose-1,5-bisphosphate regeneration capacity per unit of leaf-N content. *Plant, Cell and Environment* **26**, 255–263.

Takashima T., Hikosaka K. & Hirose T. (2004) Photosynthesis or persistence: Nitrogen allocation in leaves of evergreen and deciduous Quercus species. *Plant, Cell and Environment* **27**, 1047–1054.

Tazoe Y., Von Caemmerer S., Badger M.R. & Evans J.R. (2009) Light and CO2 do not affect the mesophyll conductance to CO2 diffusion in wheat leaves. *Journal of Experimental Botany* **60**, 2291–2301.

Théroux-Rancourt G. & Gilbert M.E. (2017) The light response of mesophyll conductance is controlled by structure across leaf profiles. *Plant Cell and Environment* **40**, 726–740.

Timm S., Florian A., Arrivault S., Stitt M., Fernie A.R. & Bauwe H. (2012) Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Letters* **586**, 3692–3697.

Timm S., Wittmiß M., Gamlien S., Ewald R., Florian A., Frank M., … Bauwe H. (2015) Mitochondrial dihydrolipoyl dehydrogenase activity shapes photosynthesis and photorespiration of Arabidopsis thaliana. *The Plant Cell* **27**, 1968–1984.

Warren C.R. & Dreyer E. (2006) Temperature response of photosynthesis and internal conductance to CO2: Results from two independent approaches. *Journal of Experimental Botany* **57**, 3057–3067.

Xiong D., Douthe C. & Flexas J. (2018) Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. *Plant, Cell & Environment* **41**, 436–450.

Xiong D., Liu X., Liu L., Douthe C., Li Y., Peng S. & Huang J. (2015) Rapid responses of mesophyll conductance to changes of CO2 concentration, temperature and irradiance are affected by N supplements in rice. *Plant, Cell and Environment* **38**, 2541–2550.

Yamori W., Evans J.R. & Von Caemmerer S. (2010a) Effects of growth and measurement light intensities on temperature dependence of CO2 assimilation rate in tobacco leaves.

Plant, Cell and Environment **33**, 332–343.

Yamori W., Kondo E., Sugiura D., Terashima I., Suzuki Y. & Makino A. (2016a) Enhanced leaf photosynthesis as a target to increase grain yield: Insights from transgenic rice lines with variable Rieske FeS protein content in the cytochrome b6/f complex. *Plant, Cell and Environment* **39**, 80–87.

Yamori W., Kusumi K., Iba K. & Terashima I. (2020) Increased stomatal conductance induces rapid changes to photosynthetic rate in response to naturally fluctuating light conditions in rice. *Plant, Cell & Environment* **43**, 1230–1240.

Yamori W., Makino A. & Shikanai T. (2016b) A physiological role of cyclic electron transport around photosystem I in sustaining photosynthesis under fluctuating light in rice. *Scientific reports* **6**, 20147.

Yamori W., Nagai T. & Makino A. (2011) The rate-limiting step for CO2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C3 crop species. *Plant, Cell and Environment* **34**, 764–777.

Yamori W., Noguchi K., Hikosaka K. & Terashima I. (2010b) Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. *Plant Physiology* **152**, 388–399.

Yang Y.-J., Hu H. & Huang W. (2020) The Light Dependence of Mesophyll Conductance and Relative Limitations on Photosynthesis in Evergreen Sclerophyllous Rhododendron Species. *Plants* **9**, 1536.

Yang Y.-J., Tong Y.-G., Yu G.-Y., Zhang S.-B. & Huang W. (2018a) Photosynthetic characteristics explain the high growth rate for Eucalyptus camaldulensis: Implications for breeding strategy. *Industrial Crops and Products* **124**, 186–191.

Yang Z.-H., Huang W., Yang Q.-Y., Chang W. & Zhang S.-B. (2018b) Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between Cypripedium and Paphiopedilum, Orchidaceae. *Photosynthesis Research* **136**, 315–328.

Zhang Y., Kaiser E., Marcelis L.F.M., Yang Q. & Li T. (2020) Salt stress and fluctuating light have separate effects on photosynthetic acclimation, but interactively affect

biomass. *Plant, Cell & Environment* **43**, 2192–2206.

Table 1. Physiological characteristics of leaves from plants grown under three different nutrient concentrations (low, medium and high nitrogen). All parameters were measured at 1500 µmol photons m^{-2} s^{-1} and 400 µmol mol^{-1} CO2 concentration. Values are means ± SE (n = 5).

	Low N	Medium N	High N
Leaf N content (g m^{-2})	0.42 ± 0.03a	0.71 ± 0.3b	1.2 ± 0.07c
SPAD value	29.2 ± 1.2a	40.2 ± 1.7b	50.1 ± 1.7c
A_N (µmol m^{-2} s^{-1})	5.9 ± 0.3a	10.2 ± 0.29b	19.1 ± 0.67c
g_s (mol m^{-2} s^{-1})	0.22 ± 0.02a	0.25 ± 0.01a	0.31 ± 0.01b
g_m (mol m^{-2} s^{-1})	0.045 ± 0.002a	0.09 ± 0.007b	0.19 ± 0.01c
ETR (µmol m^{-2} s^{-1})	44 ± 2.7c	80 ± 2.0b	156 ± 3.9a

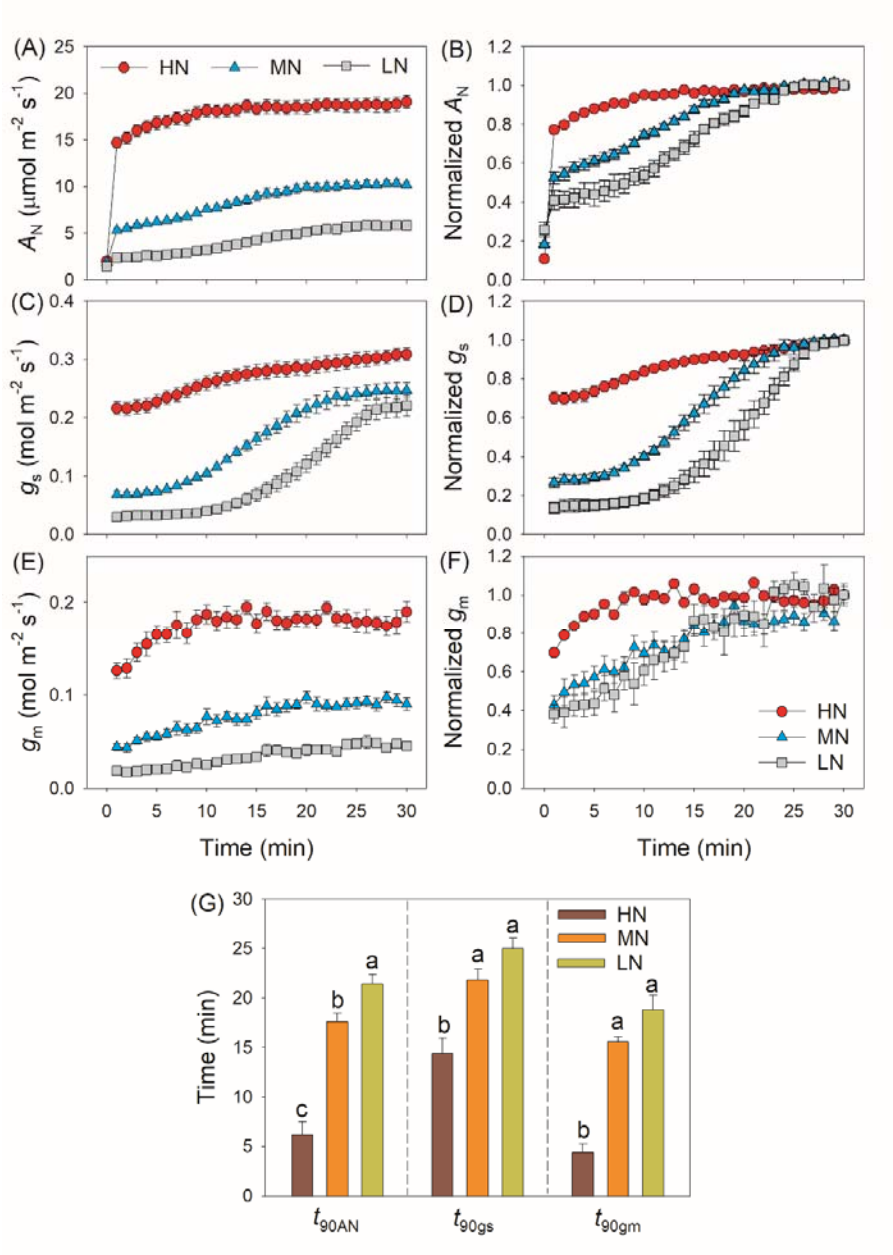

Figure 1. Induction response of net CO_2 assimilation rate (A_N), stomatal conductance (g_s) and mesophyll conductance (g_m), and the time required to reach 90% of the maximum values of A_N, g_s and g_m (t_{90AN}, t_{90gs}, t_{90gm}) after transition from 50 to 1500 μmol photons m^{-2} s^{-1}. A_N, g_s and g_m were measured every 1 min. Values are means ± SE (n = 5). Different letters indicate significant differences among different treatments. The relative A_N, g_s and g_m curves were obtained from the standardization against the maximum values after 30 min photosynthetic induction at high light. HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

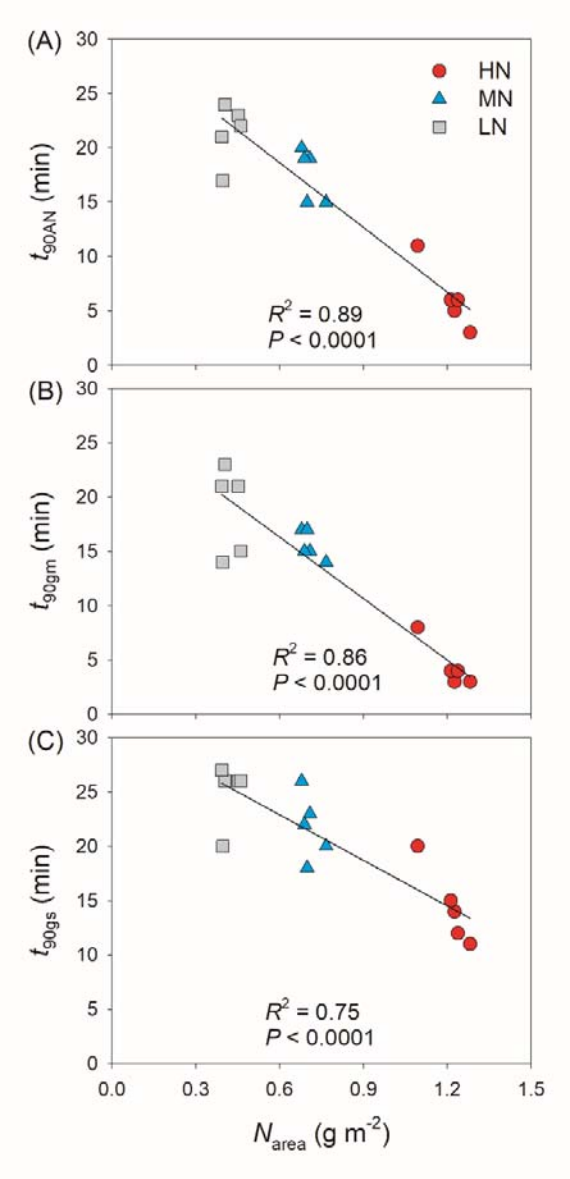

Figure 2. Effects of leaf N content on the time required to reach 90% of the maximum values of A_N, g_s and g_m (t_{90AN}, t_{90gs}, t_{90gm}) after transition from 50 to 1500 μmol photons m^{-2} s^{-1}. HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

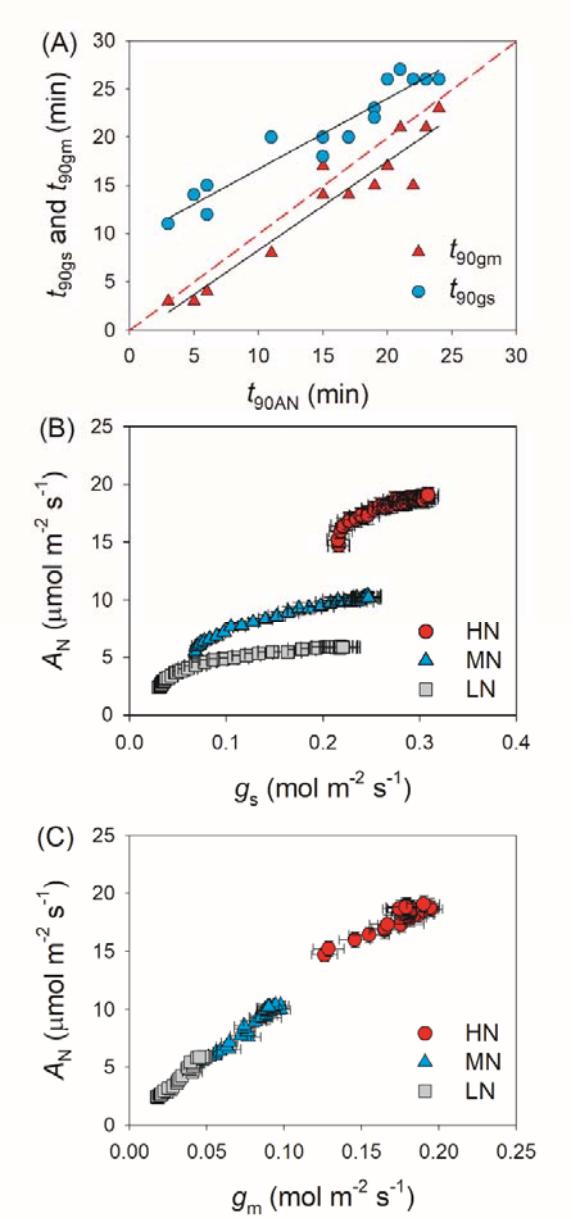

Figure 3. (**A**) Relationships between t_{90AN}, t_{90gs} and t_{90gm} after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. (**B** and **C**) Relationships between g_s, g_m and A_N after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. Values are means ± SE (n = 5). HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

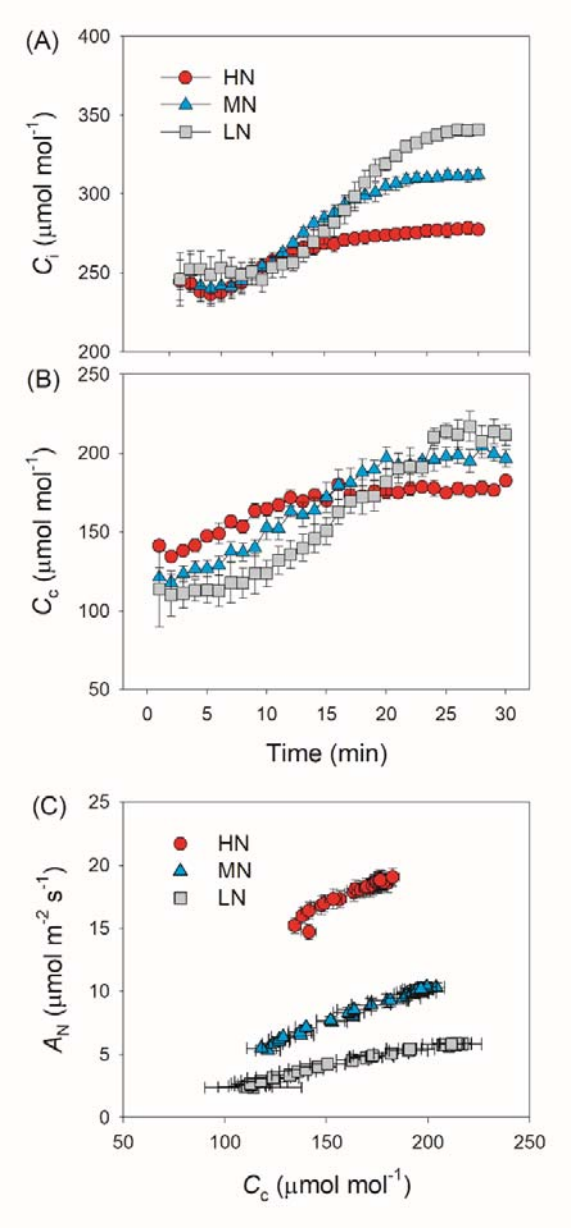

Figure 4. (**A** and **B**) Response of intercellular CO_2 concentration (C_i) and chloroplast CO_2 concentration (C_c) after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. (**C**) Relationship between C_c and A_N after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. Values are means ± SE (n = 5). HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

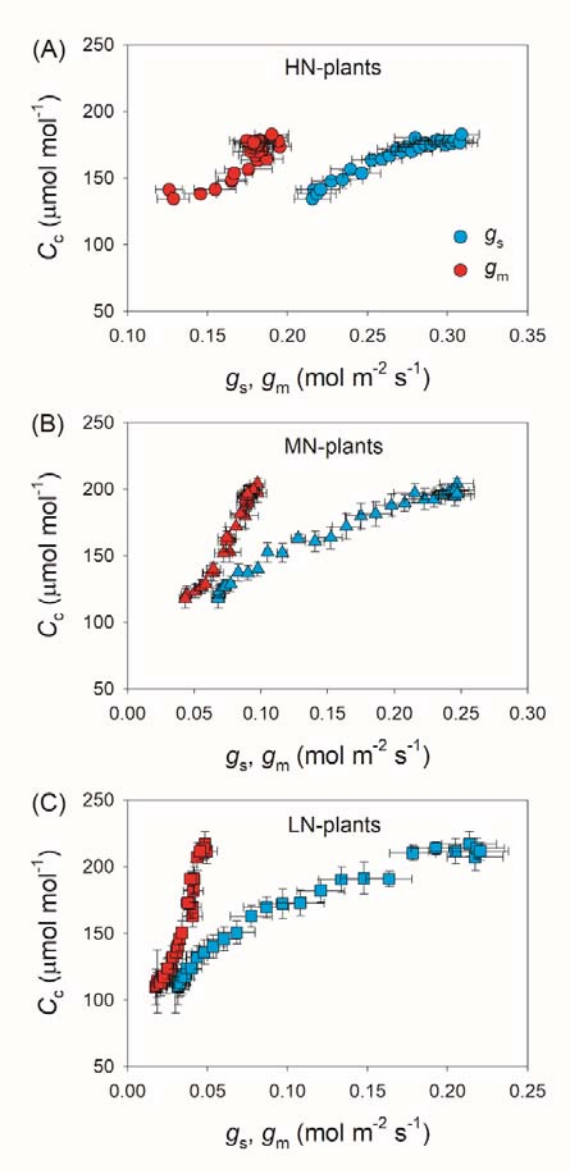

Figure 5. Relationships between g_s, g_m and C_c after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. Values are means ± SE (n = 5). HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

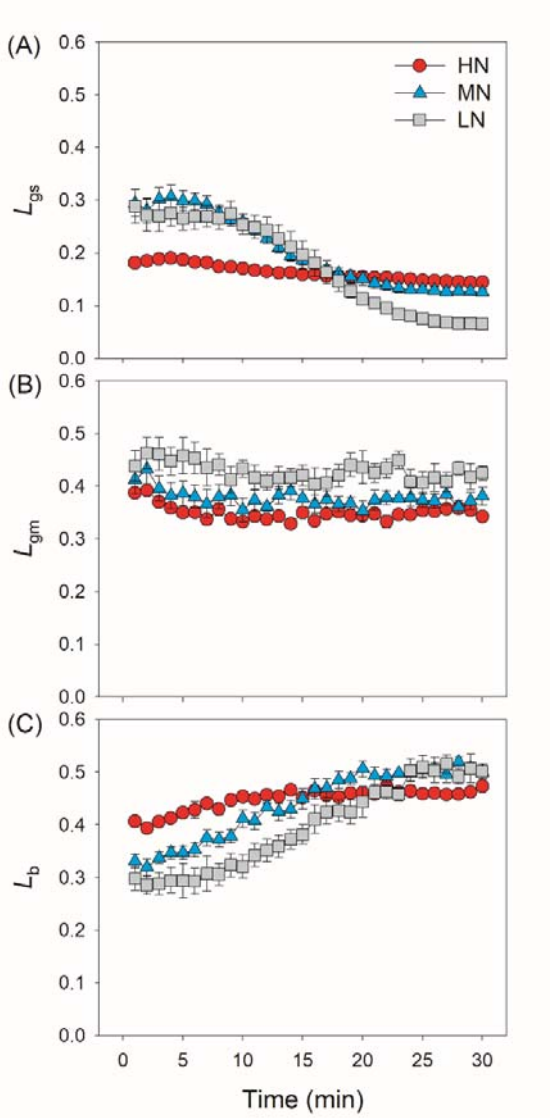

Figure 6. Quantitative analysis of the relative limitations of g_s, g_m and biochemical factors imposed to photosynthesis after transition from 50 to 1500 μmol photons m^{-2} s^{-1}. Values are means ± SE (n = 5). HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.

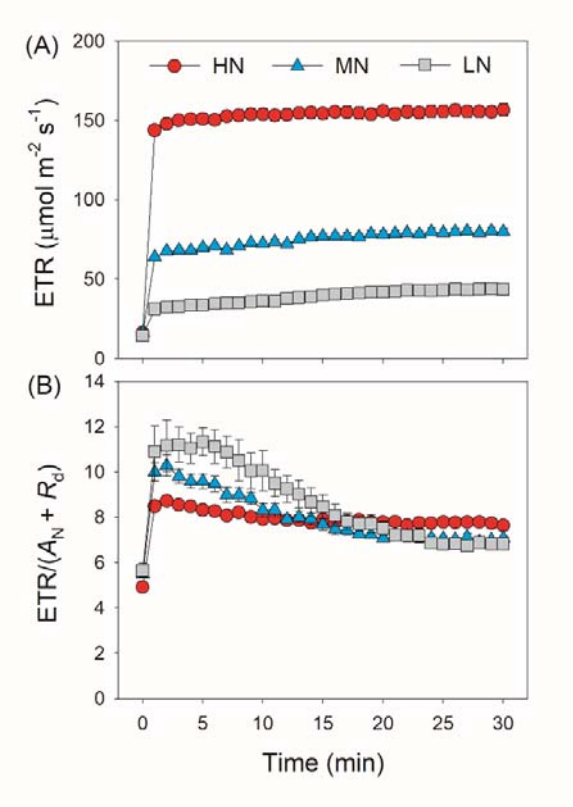

Figure 7. Response of electron transport rate (ETR) and the ratio of ETR to (A_N + R_d) after transition from 50 to 1500 µmol photons m^{-2} s^{-1}. Values are means ± SE (n = 5). HN, MN and LN represent tomato plants grown under high, medium and low N concentrations, respectively.