Photosynthesis in the fleeting shadows: an overlooked opportunity for increasing crop productivity?

Yu Wang1, Steven J. Burgess1, Elsa M. de Becker1,2 and Stephen P. Long1,3,4,*

1Carl R Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, 2Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, 3Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, and 4Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

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SUMMARY

Photosynthesis measurements are traditionally taken under steady-state conditions; however, leaves in crop fields experience frequent fluctuations in light and take time to respond. This slow response reduces the efficiency of carbon assimilation. Transitions from low to high light require photosynthetic induction, including the activation of Rubisco and the opening of stomata, whereas transitions from high to low light require the relaxation of dissipative energy processes, collectively known as non-photochemical quenching (NPQ). Previous attempts to assess the impact of these delays on net carbon assimilation have used simplified models of crop canopies, limiting the accuracy of predictions. Here, we use ray tracing to predict the spatial and temporal dynamics of lighting for a rendered mature Glycine max (soybean) canopy to review the relative importance of these delays on net cumulative assimilation over the course of both a sunny and a cloudy summer day. Combined limitations result in a 13% reduction in crop carbon assimilation on both sunny and cloudy days, with induction being more important on cloudy than on sunny days. Genetic variation in NPQ relaxation rates and photosynthetic induction in parental lines of a soybean nested association mapping (NAM) population was assessed. Short-term NPQ relaxation (<30 min) showed little variation across the NAM lines, but substantial variation was found in the speeds of photosynthetic induction, attributable to Rubisco activation. Over the course of a sunny and an intermittently cloudy day these would translate to substantial differences in total crop carbon assimilation. These findings suggest an unexplored potential for breeding improved photosynthetic potential in our major crops.

Keywords: photosynthetic induction, non-photochemical quenching, NPQ, food security, soybean, wheat, photosystem II, photoinhibition, stomata, crop breeding, leaf canopy, Rubisco activase.

INTRODUCTION

The improvement of crop photosynthesis for yield increases has focused on rates of leaf CO2 uptake at light saturation (A_sat) and under constant light. No correlation between variation in A_sat and yield has been found across a selection of modern Triticum aestivum (wheat) accessions (Rawson et al., 1983). Furthermore, wild ancestors of wheat showed higher A_sat values than elite cultivars (Dunstone et al., 1973). Such influential early findings led to skepticism that photosynthesis can be improved in crops, and this view persists for some today, despite evidence that bioengineered increases in steady-state photosynthesis do correspond to significant increases in productivity (Köhler et al., 2016; Sinclair et al., 2019; South et al., 2019). Under field conditions, however, the light in a crop canopy is rarely constant. Fluctuations result from intermittent cloud cover but more importantly from the continuous change in the angle of the sun over the course of the day, causing intermittent shadowing within the canopy by overlying leaves and other plant structures (Zhu et al., 2004; Wang et al., 2017b). Might we have overlooked a major opportunity by focusing on steady-state photosynthesis?

A dynamic light environment affects photosynthesis in two main ways. First, a leaf in the shade of another
receives about 1/10th of the light of a leaf in full sun (Zhu et al., 2004). These periods of full sun and of shade may last from seconds to hours (Zhu et al., 2004; Deans et al., 2019; Tanaka et al., 2019). Using reverse ray tracing, and assuming a random distribution of leaves, Zhu et al. (2004) showed that even on a clear day, leaves in a static canopy will experience over 20 sun–shade–sun transitions. When leaves move into full light after a period of shade, the leaf CO₂ uptake rate (A) does not instantly reach its maximum value, but rises gradually over several minutes to approach a new steady state. This has been termed photosynthetic induction, and it causes a substantial reduction in the efficiency of carbon fixation (Yamori et al., 2012; Soleh et al., 2017; Taylor and Long, 2017; Salter et al., 2019). Second, leaves in full sunlight receive more energy than they can use, leading to the activation of non-photochemical quenching (NPQ), which dissipates excess energy as heat. This reduces the production of oxidizing radicals via excited-state chlorophyll molecules that would damage the photosynthetic apparatus. Similarly, in a sun–shade transition there is a loss of potential CO₂ assimilation. This is because various components of NPQ have relaxation half-lives ranging from seconds to hours, and therefore the leaf continues dissipating light as heat even after it is moved into the shade, where it could use all of the light received in photosynthesis. This forces A below the steady-state level that it will eventually resume. Practical proof of the importance of this was shown with a bioengineered increase in the rate of dissipation of NPQ during sun–shade transitions. This resulted in a doubling of A in fluctuating light and a significant increase in Nicotiana tabacum (tobacco) productivity in replicated-plot field trials (Kromdijk et al., 2016). Many NPQ components have been identified so far, such as: energy-dependent quenching, qE (Krause et al., 1982); zeaxanthin-dependent quenching, qZ (Dall’Osto et al., 2005; Nilkens et al., 2010); chloroplast relocalization-dependent quenching, qR (Cazzaniga et al., 2013); state transition-dependent quenching, qT (Nilkens et al., 2010); photoinhibition quenching qL; and photoinhibition-independent sustained quenching qH (Malnoe et al., 2018). However, the relative contribution of these processes may depend on the conditions and species.

It has been argued that evolution and breeder selection would have already optimized photosynthesis in our crops (Winer, 2019). Two factors, with respect to photosynthesis in fluctuating light, counter this argument. First, the ancestors of our major crops evolved in relatively open habitats that could support the production of few leaves, hence both shading and self-shading would have been rare. For example, in the case of Glycine max (soybean), the wild ancestor is an annual twining vine that climbs on other stems and spaces its leaves to largely avoid shading. In contrast, modern elite cultivars have been bred as bush forms, grown at densities where they will form 5–7 m² of leaf area over every 1 m² of field. In essence, the leaves of what were sun plants by origin are now grown so that most leaves are shaded or intermittently shaded. Given the speed at which planting density has increased for our major crops, there is good reason to expect that neither evolution nor breeder selection have kept pace. Indeed, two major crops have now been shown to fail to adapt to shading to optimize canopy photosynthesis (Pignon et al., 2017). Second, current [CO₂] and light levels are co-limiting to photosynthesis in C3 crops. Atmospheric [CO₂] has risen from the 220 ppm average of the past 25 million years to 407 ppm in 2018, with half of that increase occurring in just the last 60 years. This means that light has become progressively more limiting and CO₂ has become progressively less limiting, strongly affecting photosynthetic efficiency in the shade (Long et al., 2004). Again, it is unlikely that there has been sufficient time for any adaptation to this change. This may be reflected in the large variation in speeds of induction on shade–sun transitions within the germplasm of Manihot esculenta (cassava), Oryza sativa (rice), soybean and wheat (Soleh et al., 2017; De Souza et al., 2019; Salter et al., 2019; Acevedo-Siaca et al., 2020). For example, the between-accession variation in CO₂ assimilated during the induction was three times that of the steady-state assimilation in cassava (De Souza et al., 2019), suggesting that optimization of photosynthesis in fluctuating light has not occurred.

As a result of fluctuations in light the leaf transiently forgoes potential assimilation compared with what could be achieved with an instantaneous response of photosynthesis (Zhu et al., 2004; Taylor and Long, 2017; Deans et al., 2019). Over the course of a day, how much potential photosynthesis does a crop canopy forgo? Two recent estimates predicted a daily loss for wheat of up to 15–21% (Taylor and Long, 2017; Salter et al., 2019); however, these estimates did not use realistic canopy models, simulated only small parts of the canopy and did not consider the combined effect of shade–sun and sun–shade transitions. More accurate assessments of these efficiency losses during light fluctuations require the representation of actual crops and the spatial and temporal dynamics of lighting across all leaves in the canopy.

Using soybean as an example, here the structure of an actual canopy of an elite cultivar is combined with forward ray tracing to predict the spatial dynamics of lighting across the entire canopy throughout the course of both a clear sky and an intermittently cloudy day. This dynamic lighting is combined with kinetics of both induction of photosynthesis and NPQ relaxation to quantitatively review the losses that result compared with an instantaneous return to steady-state photosynthetic rates on light transitions. To simplify the simulation, we divided the components of NPQ dynamics into two groups: short term, ≤30 min (STNPQ), and long term, >30 min (LTNPQ). The
variation measured in both induction and STNPO relaxation across parental lines of a nested association mapping (NAM) population of soybean was used to assess their value for breeding for increased speeds of adjustment to light fluctuations.

RESULTS

The light absorption of a soybean canopy was simulated for a clear sky (sunny) and intermittently cloudy (cloudy) day in August using the ray-tracing algorithm (Figure 1). The diurnal light absorption for all points in the canopy was simulated over the daylight hours of the sunny (Figure 2a) and the cloudy (Figure 2b) day. At midday on the sunny day, a point on a leaf at the top of the canopy experiences full sunlight (>1000 µmol m⁻² sec⁻¹) (Figure 2c), whereas all points lower in the canopy experience sun flecks (Figure 2e,g). Sunflecks are seen to be highly dynamic over the course of the day, particularly in the middle layers (Figure 2e). On an intermittently cloudy day, points on both the top leaves and those below are subject to large fluctuations in absorbed light (Figure 2d,f), whereas little light reaches points on the bottom layer (Figure 2f).

The net cumulative canopy assimilation ($A_c$) was calculated over the daylight hours of the sunny (Figure 3a) and the cloudy (Figure 3b) day, driven by the simulated temporal and spatial dynamics of lighting for the whole canopy. Simulations of $A_c$ compared instantaneous responses to light fluctuations with the slower responses resulting from the delays caused by photosynthetic induction (via Rubisco; $Rc_a$), STNPO (including $qE$ and $qM$) and LTNPO. This quantified the loss of potential canopy carbon fixation caused by these delays. On the sunny day, a positive rate of net total carbon assimilation was observed at 07:00 h, and a positive rate of cumulative carbon gain continued until around 18:00 h, when canopy respiration was again predicted to exceed photosynthesis (Figure 3a). Simulated differences in cumulative assimilation resulting from delays in adjustment to light fluctuations become evident around 08:00 h, and progressively amplify in cumulative carbon gain through the daylight hours (Figure 3a). The total loss resulting from these delays is 13.5%, with LTNPO accounting for the largest part of this loss (Figures 3a and 4). On the cloudy day, because the light intensity was very low in the morning (Figure 2b), the net assimilation was negative from dawn until 09:40 h, because total canopy respiration exceeded the photosynthesis until this time. Beyond this point, the simulated differences in cumulative net assimilation resulting from delays in adjustment to light fluctuations progressively amplified in cumulative net assimilation until 17:00 h (Figure 3b). The total loss as a result of these lags was 12.5%, with STNPO accounting for the largest part of this loss (Figures 3 and 4).

Although the simulated proportionate losses were similar on both days (Figure 4), the absolute loss on the intermittently cloudy day (40 mmol m⁻²) was less than half that on the sunny day (115 mmol m⁻²).

Partitioning the causes of simulated losses on the sunny day shows that resulting from LTNPO was almost twice the loss resulting from STNPO, and was approximately 2.7 times the loss caused by Rubisco activation (Figure 4). By contrast, on a cloudy day the loss resulting from STNPO is higher, contributing about 40% of the total loss, whereas the loss resulting from Rubisco induction is similar to that resulting from LTNPO (Figures 3b and 4).

Figure 1. Modelled Glycine max (soybean) canopy structure and lighting (PAR = Photosynthetically active photon flux), predicted from ray tracing for a clear sky on 20 August, at Champaign, Illinois, USA (40.11°N). Colors indicate the spatial heterogeneity of the intensity of the absorbed light at noon.
Figure 2. Panel (b) shows the recorded incident photosynthetically active photon flux on 20 August 2019, above the soybean canopy of Figure 1, which was an intermittently cloudy day. Panel (a) shows the predicted light intensity for the same day based on sun–earth geometry and an atmospheric transmittance of 0.85, assuming a cloud-free clear-sky day. The diurnal light absorption simulated for single pixels on the leaves in the top, middle and bottom of the soybean canopy on the sunny day are shown in panels (c), (e) and (g), respectively, and for the cloudy day in panels (d), (f) and (h), respectively.

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To assess the potential impact of genotypic variation on assimilation, NPQ relaxation rates were measured for the 41 parental lines of the soybean NAM population. Variation in qE and qM across the NAM population were 40% and 49% between the slowest and fastest genotype, respectively. Values for these two genotypes and the average across all members of the NAM population were used in the model. The genotype with the fastest relaxation (NAM27) assimilated about 1.3% more CO$_2$ across the day compared with the genotype with the slowest relaxation (NAM23), and assimilated approximately 0.8% more CO$_2$ than average for the sunny day (Figure 5). On the cloudy day, the fastest genotype (NAM27) assimilated approximately 0.9% more than average (Figure 5), suggesting small but significant additive effects across a growing season.

Previously published measurements of photosynthetic induction in the NAM population showed large variation between genotypes in induction (Soleh et al., 2017). The time course of Rubisco activation ($t_{\text{Rubisco}}$) for the two genotypes representing the slowest and fastest induction rates, and a third representing the middle of this range, were added here to the canopy model to simulate the variation in loss caused by the speed of the response on the sunny and the cloudy days (Figure 6). The loss in assimilation as a result of Rubisco activation is up to 17.4% for NAM8, which had the slowest Rubisco activation ($t_{\text{Rubisco}} = 1850.0$ sec), whereas NAM23, the genotype with the fastest Rubisco activation ($t_{\text{Rubisco}} = 129.7$ sec), reduced the loss to only 2.3% on the cloudy day and 1.9% on the sunny day.

**DISCUSSION**

Using a rendering of an actual fully developed field canopy (Figure 1), the present study suggests that the lower efficiency of photosynthesis during light fluctuations for an elite soybean cultivar costs about 13.5% of potential crop assimilation on both sunny and cloudy days in the critical pod-filling phase (Figure 4). On the simulated clear-sky day, LTNPQ appears to be the largest cause of this loss. On a cloudy day, photosynthetic induction and LTNPQ
contribute equally, and the loss resulting from STNPQ contributes the most, at 40% (Figure 4). Crop biomass is about 40% carbon. Assuming then that one mole of CO₂ assimilated, net of respiration, results in 75 g of biomass, then the growth loss resulting from lags in the adjustment of photosynthesis to light fluctuations in the canopy would be a substantial 90 kg ha⁻¹ day⁻¹ on a sunny day. With no mechanisms known that could allow for the instantaneous induction of photosynthesis to light fluctuations in the canopy would be a substantial 90 kg ha⁻¹ day⁻¹ on a sunny day. With no mechanisms known that could allow for the instantaneous induction of photosynthesis to shade-sun transitions or the relaxation of NPQ on sun–shade transitions, it appears unlikely that breeding or bioengineering could recover more than about half of this loss. Nevertheless, a 6.5% increase in net photosynthetic efficiency, if translated into increased crop yields, would be exceptional. It could either provide a key part of the anticipated future need for increased yield potential to ensure global food security or, should food demand stabilize, serve to reduce the global footprint of arable agriculture (Long et al., 2015). How could these losses in fluctuating light be decreased?

The engineered acceleration of STNPQ relaxation has already been undertaken in tobacco by upregulating the levels of the two enzymes involved in the interconversion of violaxanthin and zeaxanthin and the photosystem-II protein PsbS, which affects the amplitude of NPQ. This increased the quantum yield of CO₂ assimilation in fluctuating light and the rate of whole-chain electron transport by approximately 50%. In replicated field trials, biomass production was increased by 14–21%; however, this was achieved with a 50- to 100-fold increase in the two enzymes and an approximately fivefold increase in PsbS (Kromdijk et al., 2016). It would seem unlikely that this scale of diversity exists within the germplasm of a crop, suggesting that improvements on this scale might only be achieved by the transgenic addition of extra copies of the relevant genes or by the engineering of promoter regions to upregulate expression. This might be confirmed by the results obtained here with the NAM population parent lines, where the gain achieved with the fastest STNPQ-relaxing line showed only a 1% improvement over the average in terms of Aₚ (Figure 5). Although small, this could still have value. In 2017 soybean occupied 124 Mha of the global surface, which produced 353 Mt of beans (FAOStat, 2017). As such, a 0.8% increase could mean an additional 2.8 Mt or it could release over 1 Mha of land no longer needed for soybean production. Importantly, this increase, although small in relative terms, can potentially be gained by conventional breeding.

Given that the relaxation of LTNPQ is the largest contributor to losses on a sunny day, this suggests that the largest gains may be achieved by focusing on this aspect. Several genes, in particular those involved in the repair and replacement of the photosystem-II D1 protein, have been functionally implicated in the slow phase of NPQ recovery (Nixon et al., 2010). Although the optimal targets in crops remain unclear, it will be important to see these tested in model plants. With the rapid growth of complete genomic sequences for many accessions of major crops, genome-wide association analysis provides an approach to reveal further targets (Wang et al., 2017a).
What factors limit the speed of induction on a shade–sun transition? In the chloroplast, photosynthesis is limited by the build-up of Calvin cycle intermediates, in particular the CO₂ acceptor molecule ribulose 1,5-bisphosphate, and the induction of light-activated photosynthetic enzymes, in particular the activation of Rubisco by Rca. Sufficient accumulation of ribulose 1,5-bisphosphate is assumed to require approximately 60 sec, whereas the activation of Rubisco may require more than 10 min (Mott and Woodrow, 2000; Taylor and Long, 2017). At the leaf level, induction can be limited by slow stomatal opening, where full opening can require many minutes (McAusland et al., 2016; De Souza et al., 2019; Faralli et al., 2019; Acevedo-Siaca et al., 2020), and by mesophyll conductance, which generally increases with incident light. The rate of increase in mesophyll conductance upon induction is generally considered faster than both stomatal opening and Rubisco activation (Deans et al., 2019), but the variability between species and environmental conditions is not well defined. Several studies have inferred from both modeling and the in vivo estimation of Rubisco activity ($V_{c,\text{max}}$) that the activation of Rubisco is the key limitation to the speed of induction (Mott and Woodrow, 2000; Yamori et al., 2012; Soleh et al., 2016; Soleh et al., 2017; Taylor and Long, 2017). This gains support from the observation that in wheat and soybean, intercellular [CO₂] is higher during induction than at steady state (Soleh et al., 2017; Taylor and Long, 2017), whereas the converse would be expected if stomatal opening was the dominant factor. In other species the speed of stomatal opening appears to be the dominant limitation limiting the speed of induction (McAusland et al., 2016; De Souza et al., 2019), and this may be accentuated under stress conditions (Faralli et al., 2019). In the current study, only the activation of Rubisco by Rca was considered, so the losses resulting from slow induction must be considered a minimum, where slow increases in stomatal and mesophyll conductance could exacerbate these losses. There is already evidence that increasing the activity of Rca by the upregulation of expression increases the speed of activation (Yamori et al., 2012), whereas site-directed mutagenesis has been shown as a non-transgenic means to increase Rca activity (Perdomo et al., 2019). In addition, significant natural variation in the speed of induction has been demonstrated within the germplasm of cassava, soybean and wheat (Soleh et al., 2017; De Souza et al., 2019; Salter et al., 2019), providing further non-transgenic opportunities for decreasing the losses resulting from reduced photosynthetic efficiency during induction. Simulation of this variation in our canopy model suggests that considerable gains could be achieved in soybean by conventional breeding that selects for faster rates of induction (Figure 6).

The purpose of this work was to highlight the potential for improving crop photosynthesis under non-steady-state lighting conditions. To do this we had to draw on disparate sources for model parameterization, as not all parameters were available for soybean. Additionally, some of the parameters used were measured using different protocols, such as NPQ relaxation kinetics of soybean cv. LD11-2170 and the NAM population. Therefore, the predictions here should be taken only as an indication of opportunity, and need to be further improved by better measurements and parametrization. First, the rate constant of the NPQ relaxation is possibly related to light intensities or light-intensity differences (Dall’Osto et al., 2014). Second, it is not clear how leaf position and age influence the NPQ relaxation speed. Third, the long-term NPQ responses of soybean and most major crops have not been well defined, and the correlation between LTNPQ and the accumulated light input has not been validated with sufficient data. Fourth, the correlation between Rubisco activation kinetics and light intensity is poorly defined. Thus more integrated measurement and analysis of NPQ relaxation, Rubisco activation kinetics, stomatal opening and mesophyll conductance dynamics in the major crops will improve the model prediction and ability to partition causes. Finally, the lighting was simulated for 5-mm² pixels of the leaf surface; however, NPQ activation and Rubisco activation occur at the level of the chloroplast, with a cross-sectional area of about 25 μm². As a result, the speed of the light change at an individual chloroplast with the change in solar angle will be faster than that simulated. Simulating smaller areas greatly increases the computational time, but is likely to become practical as computational power increases. The compromise used here will have resulted in some underestimation of the cost of these light transitions, however.

In conclusion, this first model analysis of light fluctuations in a rendering of an actual crop canopy indicates unexploited breeding and bioengineering targets to substantially improve photosynthetic efficiency and productivity. Although this analysis is limited to soybean, it appears reasonable to expect similar gains in other C₃ crops, including cassava, wheat and rice. Although we cannot be certain that an improvement in photosynthetic efficiency will increase the yield, there is evidence for all four crops that an artificial increase in leaf photosynthesis by season-long elevation of [CO₂] under open-air field conditions results in highly significant increases in yield (Long et al., 2006; Rosenthal et al., 2012; Hasegawa et al., 2013).

**EXPERIMENTAL PROCEDURES**

**3D soybean model and light distribution simulations**

The dynamics of lighting within a soybean canopy were predicted with a 3D architectural representation, using our previously presented framework for crop canopies (Song et al., 2013; Wang et al., 2017b). The model was parameterized on the measured architecture of a soybean crop (G. max L. Merr., Pioneer 93B15) on the University of Illinois South Farms. The leaf lengths, widths, petiole lengths...

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and angles for trifoliate leaves of soybean (Aug 20) were obtained from Song et al., (2019). The canopy had a row spacing of 38 cm and a plant spacing within the rows of 10 cm. The model divides the surface of each leaf into ‘pixels’ of approximately 5 mm² in area, which is much smaller than the ‘pixels’ used in Song et al., (2019; about 1 cm²). To estimate light changes in the canopy, a forward ray-tracing algorithm (FASTRACER; Song et al., 2013; Wang et al., 2017b; Townsend et al., 2018; Song et al., 2019) was used to predict the light absorption of each 5-mm² pixel every 10 sec from 05:00 to 19:00 h on 20 August 2019 in Champaign, IL, USA (40.11 N, 88.21 W). At each time point the direct and diffuse light entering and within the canopy is predicted, together with the scattered radiation through reflection from, and transmission through, the leaves within the canopy. Leaf reflectance and transmission were both set to 0.075 (Song et al., 2013). The sum of the direct, diffuse and scattered light incident at each pixel, less the reflectance and transmission, gave the absorbed photosynthetically active photon flux. Atmospheric transmittance was set as 0.85 to estimate the incident light absorption of each 5-mm² leaf pixel on the cloudy day, Leaf reflectance and transmission were both set to 0.075 (Song et al., 2013). The sum of the direct, diffuse and scattered light incident at each pixel, less the reflectance and transmission, gave the absorbed photosynthetically active photon flux. Atmospheric transmittance was set as 0.85 to estimate the incident direct and diffuse light reaching the top of the canopy at each time point on a sunny day (Song et al., 2013). A partially cloudy day was simulated by using the measured direct and diffuse light on 20 August 2018 in Bondville, IL, USA (SURFRAD, 2019). For each time point (t), the light absorption of each 5-mm² leaf pixel on the cloudy day, \( I_{abs}(t) \), was calculated as:

\[
  I_{abs}(t) = \frac{Q_d(t)}{Q_{solar}}, \tag{1}
\]

where \( I_{abs}(t) \) is the light absorption of each leaf pixel on the sunny day, which was calculated by the ray-tracing algorithm. \( Q_{solar}(t) \) is the incident light above the canopy on the cloudy day and \( Q_{solar}(t) \) is the incident light above the canopy on the cloudy day, based on the actual incident light measured.

### Simulation of dynamic photosynthesis

Dynamic photosynthetic rates were calculated for every 10 sec (\( \Delta t \)) of the day using the absorbed light for each 5-mm² pixel, considering the rates of Rubisco activation and NPO relaxation. Rubisco deactivation was also taken into account, as the extent of the deactivation during a period of shade affects the duration of the induction following a return to higher light levels. The in vivo dynamics of Rubisco activation were calculated using the Rca model of Mott and Woodrow (2000). The steady-state maximum Rubisco activity (\( V_{xmax} \)) was related to the level of Rca (70 mg m⁻¹) (Mott and Woodrow, 2000), where \( K_{plca} \) is a constant in determining the maximum steady-state activity of Rubisco (Table 1):

\[
  V_{max}@s = \frac{V_{max}@0[Rca]}{K_{plca} + [Rca]}, \tag{2}
\]

The maximum Rubisco activity (\( V_{max} \)) was calculated by the following equations:

\[
  V_{max}@s(t) = V_{max}@0@Rca \frac{I_c(t)}{I_{light} + I_c(t)}, \tag{3}
\]

\[
  A_{vmax}(t) = A_{vmax}(t) - \Delta t \left( A_{vmax}(t) - V_{max}@s(t) \right) \frac{1}{T_{rubisco}}, \tag{4}
\]

\[
  V_{max}(t) = \frac{A_{vmax}(t) \cdot V_{max}@0}{}, \tag{5}
\]

where \( t \) is time (s), \( I_c(t) \) is the absorbed light of each leaf unit at time \( t \) and \( K_{plight} \) are constants (Table 1), \( T_{rubisco} \) is the time constant of Rubisco activation and deactivation; here, \( T_{rubisco} \) is 180 sec for Rubisco activation and 300 sec for Rubisco deactivation (Taylor and Long, 2017). \( Av_{max}(t) \) is the activated proportion of Rubisco.

The short-term relaxation kinetics of non-photochemical quenching (STNQ) was defined as the dynamics of NPQ relaxation over the first 30 min, and is described by a bi-exponential curve (Dall’Osto et al., 2014). Here, \( QE \) is the fast relaxing energy-dependent quenching, whereas \( QM \) combines all of the remaining processes up to 30 min:

\[
  \Phi_{NPQs}(t) = N_1 I(t) + N_0 \tag{6}
\]

\[
  \Phi_{NPQs}(t) = \Phi_{NPQs}(t - \Delta t) - \Delta t \left( \Phi_{NPQs}(t - \Delta t) - \Phi_{NPQs}(t) \right) \tag{7}
\]

\[
  \Phi_{NPQs}(t) = \Phi_{NPQs}(t - \Delta t) - \Delta t \left( \Phi_{NPQs}(t - \Delta t) - \Phi_{NPQs}(t) \right) \tag{8}
\]

\[
  \Phi_{NPQs}(t) = \Phi_{NPQs}(t) + \Phi_{NPQs}(t) \tag{9}
\]

where \( \Phi_{NPQs} \) is the steady state \( \Phi_{NPQs} \) obtained from the measured light-response curves (see next section) of soybean (cv. LD11-2170). \( N_1 \) and \( N_0 \), which are set to 0.00028 and 0.371, are constants calculated from the measured \( \Phi_{NPQs} \). \( \Phi_{NPQs} \) and \( \Phi_{NPQs} \) are the proportions of \( QE \) and \( QM \). \( \Phi_{NPQs} \) and \( \Phi_{NPQs} \) are the time constants of \( QE \) and \( QM \) relaxation, respectively.

Long-term non-photochemical quenching (LTNQ), which includes, among other terms, photoinhibitory quenching (qI) and photoinhibition-independent sustained quenching (qH) decreases the maximum quantum yield of photosystem II (PSII) and in turn the maximum quantum yield of CO₂ uptake (Zhu et al., 2004). Following Zhu et al. (2004), the LTNQ was correlated with a weighted light dose, and the change in the maximum quantum yield of PSII photochemistry, \( \Phi_{PSII} \), was calculated as:

\[
  T_f = 0.00337^2 - 0.1795T + 3.4257, \tag{10}
\]

\[
  I_{int}(t) = \sum_{i=1}^{60} \frac{I(t - i)}{1 + \frac{i}{60}}, \tag{11}
\]

\[
  A_{Fv/Fm}(t) = 1 - I_{int}(t) \frac{T_f}{T_f}, \tag{12}
\]

where \( I_{int} \) is the weighted light dose at time \( t \). \( T_f \) is an empirical factor relating the relative decrease of \( Fv/Fm \) to temperature (\( T \)). \( A_{Fv/Fm}(t) \) was set as 25°C. \( f_s \) is an empirical constant used in determining the decrease of \( Fv/Fm \) for a given \( I_{int} \). For cold-susceptible species, \( f_s \) is 5.13.

The quantum yield of PSII was calculated as:

\[
  \Phi_{PSII} = A_{Fv/Fm}(t) \frac{Fv/Fm - \Phi_{NPQs}}{2 \Phi_{NPQs}}, \tag{13}
\]

The electron transport rate, and in turn the rate of leaf CO₂ uptake (\( A \)), is limited by the quantum yield of PSII, incident light and maximum electron transport capacity (\( U_{max} \)):

\[
  J = \min \left( \frac{1}{2} \Phi_{PSII}(t) J_{max} \right). \tag{14}
\]

The rate of leaf CO₂ uptake (\( A \)) at time \( t \) was simulated by the following equations:

\[
  A_t = \frac{(C_i - \Gamma)(J_t)}{4C_i + 9.31} - R_d, \tag{15}
\]
Table 1 Input parameters of the Glycine max (soybean) canopy model

| Parameter   | Full name                                      | Value      | Reference               |
|-------------|------------------------------------------------|------------|-------------------------|
| $C_1$       | Intercellular CO$_2$ concentration             | 280 µbar   | Environmental input     |
| $f_n$       | Empirical constant in determining the decrease | 5.1e+5     | Zhu et al. (2004)       |
| $F_{v}/F_m$ | Maximum quantum yield of photosystem II        | 0.391      | Measured                |
| $I^*$       | The CO$_2$ compensation point in the absence   | 38.6 µbar  | Von Caemmerer (2000)    |
| $J_{max}$   | Maximum electron transport capacity            | 200 µmol m$^{-2}$ s$^{-1}$ | Bernacchi et al. (2005) |
| $K_D$       | Michaelis–Menten constant of Rubisco for O$_2$ | 404 mbar   | Von Caemmerer (2000)    |
| $K_E$       | Michaelis–Menten constant of Rubisco for CO$_2$ | 248 µbar   | Von Caemmerer (2000)    |
| $k_{qRac}$  | A constant for the calculation of maximum      | 12.4 m$^{-2}$ | Mott and Woodrow (2000) |
| $k_{light}$ | A constant for the calculation of steady-state | 120 µmol m$^{-2}$ s$^{-1}$ | Sage and Seemann (1993) |
| $k_{TaoRac}$| A constant for the calculation of the time     | 214 min m$^{-2}$ | Mott and Woodrow (2000), Soleh et al. (2016) |
| $O_2$       | Intercellular O$_2$ concentration              | 210 mbar   | Environmental input     |
| $P_{qE}$    | Proportion of the contribution of qE in steady-state | 0.7        | Measured                |
| $P_{qM}$    | Proportion of the contribution of qM in steady-state | 0.3        | Measured                |
| $[\text{Rac}]$ | Concentration of Rubisco activase              | 70 mg m$^{-2}$ | Mott and Woodrow (2000) |
| $R_{d0}$    | Dark respiration                               | 1.2 µmol m$^{-2}$ s$^{-1}$ | Measured |
| Temp        | Leaf temperature                               | 25°C       | Environmental input     |
| $\tau_{qE}$ | Time constant of qE relaxation                 | 0.56 min   | Measured                |
| $\tau_{qM}$ | Time constant of qM relaxation                 | 16.8 min   | Measured                |
| $V_{\text{max0}}$ | Maximum Rubisco activity                      | 120 µmol m$^{-2}$ s$^{-1}$ | Bernacchi et al. (2005) |

Table 2 Measured non-photochemical quenching (NPQ) relaxation parameters of Glycine max (soybean) cv. LD11-2170

| Parameters             | Soybean       |
|------------------------|---------------|
| $F_{v}/F_m$            | 0.801 ± 0.032 |
| $\Phi_{qE}$            | 0.318 ± 0.016 |
| $\Phi_{qI}$            | 0.097 ± 0.015 |
| $\Phi_{qM}$            | 0.026 ± 0.009 |
| $\tau_{qE}$ (min)      | 0.558 ± 0.051 |
| $\tau_{qM}$ (min)      | 16.826 ± 9.134|

where $A(t)$ is the CO$_2$ uptake rate of a leaf pixel, $S_i$ is the surface area of each pixel and $S_{\text{ground}}$ represents the occupied ground area of the simulated canopy. All simulations were conducted in MATLAB 2017 (Mathworks®, https://uk.mathworks.com).

Measurement of dynamic NPQ parameters

To determine the kinetics of SNPQ relaxation in soybean (parameters listed in Table 2), chlorophyll fluorescence, and gas exchange were measured during the transition from high light (1800 µmol m$^{-2}$ s$^{-1}$) to low light (200 µmol m$^{-2}$ s$^{-1}$). Measurements were taken on 9 March 2019 in a controlled-environment glasshouse at the University of Illinois at Urbana-Champaign. The air temperature inside the glasshouse was set as 28°C (day)/24°C (night). Leaf CO$_2$ uptake and modulated chlorophyll fluorescence of the youngest fully expanded leaf was measured on 30-day-old soybean plants (cv. LD11-2170) with a gas-exchange system incorporating a controlled-environment leaf cuvette with a head containing a modulated chlorophyll fluorometer and an LED light source (LI-6400XT and LI-6400-40; LI-COR, https://www.licor.com). The measurements were made on six replicate plants. The leaves were first acclimated to dark for 30 min, with a leaf cuvette temperature ($T_{\text{cuvee}}$) of 28°C and a [CO$_2$] of 400 µmol mol$^{-1}$; the light intensity was then increased to 1800 µmol m$^{-2}$ s$^{-1}$ for 30 min and then decreased to 200 µmol m$^{-2}$ s$^{-1}$ for the next 30 min, to simulate a sun-shade transition. Leaf CO$_2$ exchange and modulated chlorophyll fluorescence were recorded before the light was turned on, and then every 60 sec for the following 30 min. The measured time series data of NPQ changes, represented by the following equation (Dall’Osto et al., 2014):
\[ \Phi_{\text{NQ}} = \Phi_{\text{m}} + \Phi_{\text{a}} e^{-\left(\frac{-t}{\tau_{\text{a}}}\right)} + \Phi_{\text{d}} e^{-\left(\frac{-t}{\tau_{\text{d}}}\right)}. \]  

were fitted with a polynomial (fit function: MATLAB 2017). The fitted values are listed in Table 2.

For light-response curves, the leaf was dark-adapted for 30 min and then acclimated to a light intensity of 1500 \(\mu\text{mol m}^{-2}\text{ sec}^{-1}\) and a \(\text{CO}_2\) concentration of 400 \(\mu\text{mol mol}^{-1}\) inside the cuvette. After 20 min the chamber light was varied according to the following sequence: 2000, 1500, 1000, 500, 300, 200, 100 and 50 \(\mu\text{mol m}^{-2}\text{ sec}^{-1}\). Chlorophyll fluorescence and gas-exchange measurements were recorded after the conditions inside the cuvette regained stability at each light level, the minimal time interval was 5 min. Chlorophyll fluorescence measurements were used to calculate steady-state NPQ (\(\Phi_{\text{NQ}}\), eqn 6).

**Variation in the rate of recovery of STNPQ quenching across the parents of the soybean NAM population**

The 41 parents of the soybean NAM population (Song et al., 2017; Diers et al., 2018) were planted on the South Farms of the University of Illinois at Urbana-Champaign, on 6 June 2019. The parents were planted in 1.2-m-long single-row plots with a 0.75-m row spacing. The experiment was arranged with a randomized complete block design with five replicate plots per genotype. On the 26 July 2019, when plants were at the R1 developmental stage, three 0.48-mm leaf disks were collected from the uppermost mature leaf of each replicate plot in the field and floated on water in 24-well plates for transport back to the laboratory. Leaf disks were then laid adaxial side up on square Petri dishes containing damp filter paper, sealed, wrapped in aluminum foil and incubated overnight at 25°C to allow for the complete relaxation of NPQ. The next day, the modulated PSII chlorophyll fluorescence of the disks on each Petri dish was imaged (FluorCam FC 800-C; PSI-Photon Systems Instruments, Drasov, Czech Republic, https://psci.cz/). Disks were exposed to 10 min of approximately 1000 \(\mu\text{mol m}^{-2}\text{ sec}^{-1}\) white light (6500 K) to induce NPQ, and then 50 min of darkness. Saturating pulses (4000 \(\mu\text{mol m}^{-2}\text{ sec}^{-1}\) white light) to determine \(F_m\) were given at 9, 40, 60, 80, 100, 120, 160, 200, 240, 300, 360, 420, 480, 540 and 598 sec after the actinic light was turned on, and at 1, 2, 4, 6, 8, 10, 14, 18, 22, 26, 32, 38, 44 and 50 min after the actinic light was turned off. The background was excluded automatically and NPQ values at each pulse were calculated. The maximum NPQ value for each leaf disk was set to 1, with other values normalized relative to this. Relaxation of NPQ with time was fitted by nonlinear least squares.

**Simulating the effect of variation in photosynthetic induction across the NAM parent lines**

Measured time courses of Rubisco (\(V_{\text{m}}\)) activation during photosynthetic induction were extracted from Soleh et al. (2017) for the NAM parent lines, using image-capture and analysis software (GETDATA GRAPH DIGITIZER, obtained from S. Fedorov, http://getdata-graph-digitizer.com). Fifteen points were captured per curve for each leaf disk and then fitted to an exponential function:

\[ V_{\text{m}} = a e^{\left(\frac{-t}{\tau_{\text{Rubisco}}}\right)} + c, \]  

where \(\tau_{\text{Rubisco}}\) is the time constant of Rubisco activation, \(c\) is the Rubisco activity in the dark and \(a\) is the increase of Rubisco activity from dark to light. The fitted values are listed in Table 3. Then the measured \(\tau_{\text{Rubisco}}\) values for NAM23, RC and NAM8 were used in this simulation. The time constant of Rubisco deactivation was assumed to be double the time required for the activation of each genotype (Taylor and Long, 2017).

| Parameters          | NAM23 | RC  | NAM8 |
|---------------------|-------|-----|------|
| \(a (\mu\text{mol m}^{-2}\text{ sec}^{-1})\) | 72.2  | 80.0| 85.0 |
| \(c (\mu\text{mol m}^{-2}\text{ sec}^{-1})\) | 21.6  | 17.3| 7.6  |
| \(\tau_{\text{Rubisco}}\) (sec) | 129.7 | 471.7| 1850.0|
| \(R^2\)           | 0.982 | 0.977| 0.976|

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**AUTHOR CONTRIBUTIONS**

SPL, YW and SJB designed the study. YW performed the computational analysis. YW, EMBD and SJB conducted the STNPQ measurements. SPL, YW, SJB and EMBD wrote the article.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**DATA AVAILABILITY STATEMENT**

The code and data are available at https://doi.org/10.13012/B2IDB-9453481_V1.

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