Photosynthesis | Photosynthetic Efficiency Improvement

Rebecca A Slattery and Donald R Ort, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Glossary

Conversion efficiency (ε_c) The efficiency with which plants convert light energy into biomass, also termed photosynthetic efficiency.

Guard cells Cells surrounding stomata that control pore aperture.

Mesophyll conductance (g_m) Rate of CO_2 diffusion from the leaf intercellular space into the mesophyll chloroplast.

Non-photochemical quenching (NPQ) A photoprotective process in which excess light energy is dissipated as heat.

Photorespiration Rubisco-catalyzed oxygenation of RuBP.

Stomata Pores in the leaf epidermis that allow gas diffusion into/out of the leaf.

Stomatal conductance (g_s) Rate of CO_2 diffusion from the outside air through the stomata into the leaf intercellular space.

Introduction

Current Estimates of Photosynthetic Efficiency

As the human population increases, so will the need for producing more food and feed. The global human population is estimated to reach nine billion by the year 2050, and rising affluence in some countries is leading to greater consumption of grain-fed livestock products. Crop production per land area will need to increase 60%–120% from 2005 levels to keep pace with the increasing population and rising affluence (Tilman *et al.*, 2011). Between 1961 and 2005, total crop production increased by 162%, partly due to agricultural land expansion (27%) but mostly due to intensification of agricultural practices (135%) like those initiated during the Green Revolution that led to higher yields per land area (Burney *et al.*, 2010). However, recent studies report the rate of increase in production is stagnating in several important food crops (Ray *et al.*, 2013), and increasing yield gains may be further challenged by facets of global climate change. Therefore, new and sustainable methods of improving crop productivity will be required to increase crop production to ensure sufficient nourishment for the global human population in the coming decades.

The yield potential (Y_p) of crop plants is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging, and other stresses effectively controlled. It can be calculated as $Y_p = 0.487 S_t \cdot \varepsilon_i \cdot \varepsilon_c \cdot \varepsilon_p$ (Monteith, 1977). Y_p is ultimately constrained by the amount of solar radiation available during the growing season (S_t) of a given crop in its growing region, but only approximately half of that (0.487) is within the visible spectrum (i.e., photosynthetically active radiation (PAR); 400–700 nm) that drives plant photosynthesis. S_t is acted on by three genetically determined traits of the plants. The efficiency with which plants intercept the available radiation (ε_i) is already nearing the theoretical maximum, as is the efficiency with which plants partition biomass into harvestable product (ε_p). This leaves ε_c, the efficiency with which plants convert light energy into biomass, or photosynthetic efficiency, as the only factor with substantial room left for improvement. For C3 plants at prevailing temperature and carbon dioxide levels, the theoretical maximum ε_c based on available S_t is 4.6%, and the theoretical maximum ε_c is higher in C4 plants at 6.0% (Zhu *et al.*, 2010). Indeed, the average realized ε_c of relatively unstressed major crops is less than half the theoretical maximum for major plant types in relatively non-limiting conditions (**Fig. 1**; Slattery and Ort, 2015) and is much lower in the presence of biotic and abiotic stresses. Thus, photosynthetic efficiency is a target of opportunity for improvement to increase yields, especially in important crops (Simkin *et al.*, 2019).

ε_c is determined by the efficiency of photosynthesis in both the leaf and canopy. In the leaf, the light reactions capture a portion of light energy as chemical energy in the form of NADPH and ATP. The formation of NADPH occurs as electrons are energized by light within the reaction centers to remove electrons from water that flow through a series of redox-active proteins and compounds associated with the thylakoid membrane to ultimately reduce NADP+ to NADPH (see below for more detail). This flow of electrons also results in accumulation of protons in the lumen within the thylakoid membrane, which results in both a H^+ concentration and an electrical charge difference between the lumen and stroma, termed the proton motive force (PMF). As protons flow from higher concentration and low pH in the lumen to lower concentration and high pH in the stroma through the ATP synthase enzyme, ATP is formed from ADP and inorganic phosphate. The main fate of this photosynthetically produced ATP and NADPH is to power the reactions of the Calvin-Benson cycle where CO_2 from the air is first bound to a 5-carbon sugar, ribulose-1,5-bisphosphate (RuBP), by RuBP carboxylase/oxygenase (Rubisco). For every six CO_2 molecules fixed by Rubisco there is the net production of two 3-carbon triose phosphates, which are converted to sugars and starch to be used for growth and maintenance of the plant, and the net regeneration of a RuBP molecule to continue the cycle. The sum of all leaf photosynthesis corrected for respiration (net photosynthesis) determines the overall canopy photosynthesis.

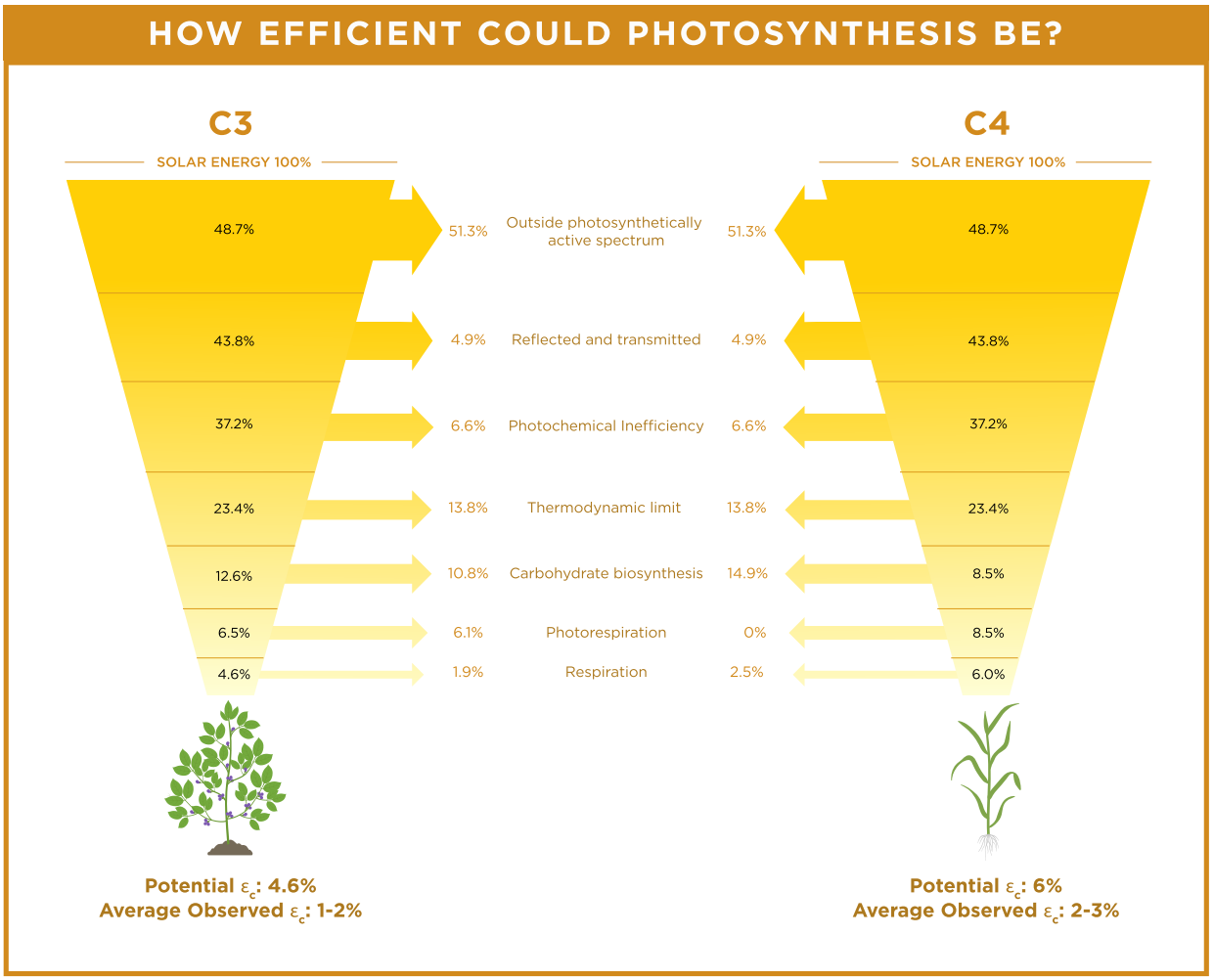

Fig. 1 Theoretically possible versus average realized photosynthetic efficiency (ε_c) in C3 and C4 crops in non-limiting conditions. Based on 100% available solar radiation incident on a crop canopy (top), inefficiencies in photosynthesis (center) limit the theoretical maximum ε_c to 4.6% in C3 crops (left) and 6.0% in C4 crops (right), whereas average observed values are even lower. Modified from Zhu, X.-G., Long, S.P., Ort, D.R., 2010. Improving photosynthetic efficiency for greater yield. Annual Review of Plant Biology 61, 235–261. Slattery, R.A., Ort, D.R., 2015. Photosynthetic energy conversion efficiency: Setting a baseline for gauging future improvements in important food and biofuel crops. Plant Physiology 168, 383–392.

Limitations to Photosynthesis

Leaf photosynthesis is limited by different factors, depending on the prevailing environmental conditions. In low light, photosynthesis is limited by the rate of light energy conversion to chemical energy via the electron/proton transport chain in the thylakoid membrane and responds linearly with increasing light intensity (Fig. 2(A), photosynthetic light-response curve). However, leaf photosynthesis often saturates at light levels far below those available on a clear sunny day in the field. For C3 crops, only about 25% of sunlight available on a cloudless summer day is needed to saturate leaf photosynthesis due to other downstream kinetic limitations. Photoprotective processes, such as non-photochemical quenching (NPQ), dissipate excess light energy, preventing photodamage while allowing for sustained high rates of photosynthesis at high light but can lead to lower than optimal rates if photoprotection remains engaged when light levels decline. If the leaf is unable to dissipate excess light as heat, photosynthetic machinery and membranes can be damaged by the excess light energy, which is termed photodamage and results in lower photosynthetic rates at any light level until the damage can be repaired, often requiring *de novo* synthesis of replacement proteins.

In C3 leaves under saturating light levels, photosynthesis is normally limited by the carboxylation rate of RuBP by the enzyme Rubisco due to insufficient CO_2, insufficient activated Rubisco, and/or insufficient RuBP. At low CO_2 concentrations, limited CO_2 substrate and/or Rubisco activity limits photosynthesis (Fig. 2(B), Rubisco-limited portion of the C3 photosynthetic CO_2-response curve). As CO_2 increases, the carboxylation reaction is instead normally limited by the availability of RuBP (Fig. 2(B), RuBP-limited portion of the photosynthetic CO_2-response curve), which is determined by the rate of RuBP regeneration in the Calvin-Benson cycle, which in turn can be limited by electron transport-driven ATP and NADPH production. At even higher CO_2 concentrations, photosynthesis is primarily limited by the rate of starch and sucrose synthesis from triose phosphates, which determines the

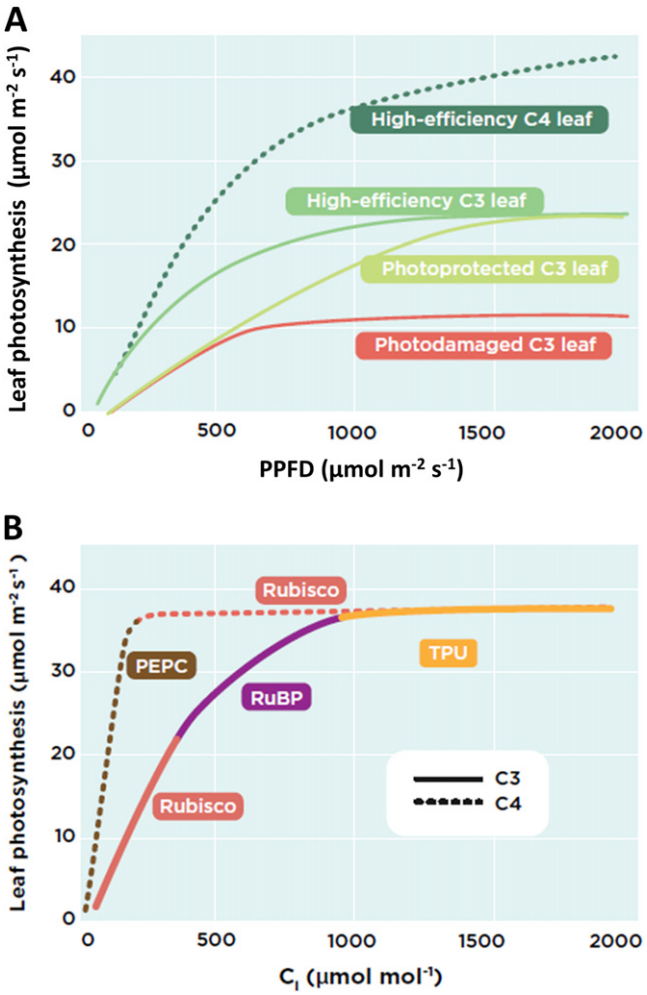

Fig. 2 Limitations to C3 and C4 photosynthetic light and CO_2 responses. A. Photosynthetic light-response in C3 and C4 leaves. The C3 curves represent the photosynthetic rates of a leaf in a high-efficiency state, a photoprotected state, and a photodamaged state. B. Photosynthetic CO_2-response curve for C3 and C4 leaves showing the underlying processes limiting photosynthetic rate with CO_2. Modified from Yin, X., Struik, P.C., 2009. C3 and C4 photosynthesis models: An overview from the perspective of crop modeling. NJAS - Wageningen Journal of Life Sciences 57, 27–38.

availability of inorganic phosphate for ATP production (**Fig. 2(B)**, triose phosphate utilization (TPU)-limited portion of the C3 photosynthetic CO_2-response curve). Typically, C3 leaves operate under Rubisco-limited photosynthesis, but as atmospheric CO_2 concentrations increase, photosynthesis will more often be RuBP limited. In C4 plants, the limitations differ due to the CO_2 concentrating mechanism whereby phosphoenolpyruvate (PEP) carboxylase (PEPC) initially fixes CO_2 in the mesophyll cell and transports the CO_2 to site of Rubisco in the bundle sheath cell in the form of a C4 dicarboxylic acid where it is decarboxylated, releasing the CO_2 initially fixed in the mesophyll. Thus, PEPC-limited photosynthesis occurs at very low CO_2 and Rubisco-limited photosynthesis occurs at higher CO_2 concentrations (**Fig. 2(B)**, C4 photosynthetic CO_2-response curve). However, both PEPC- and Rubisco-limited C4 photosynthesis can be affected by the activity of either enzyme as well as electron transport-driven regeneration of either PEP or RuBP (Yin and Struik, 2009). At current ambient CO_2 conditions C4 photosynthesis is generally Rubisco-limited, but whether the root cause is enzyme or substrate driven depends on growth conditions. At the canopy level, photosynthesis in both C3 and C4 species is often a function of light distribution within the canopy and is often limited by light in the lower canopy while experiencing light in excess of photosynthetic capacity in other parts. These major areas of limitation and opportunities for improvement are discussed in more detail below.

Carboxylation

Carbon Supply

During photosynthesis, CO_2 must travel from the air outside the leaf to the site of Rubisco carboxylation within the chloroplast stroma (**Fig. 3(A)**). The first step involves diffusion of CO_2 from the air, where the concentration of CO_2 (C_a) is relatively high, to

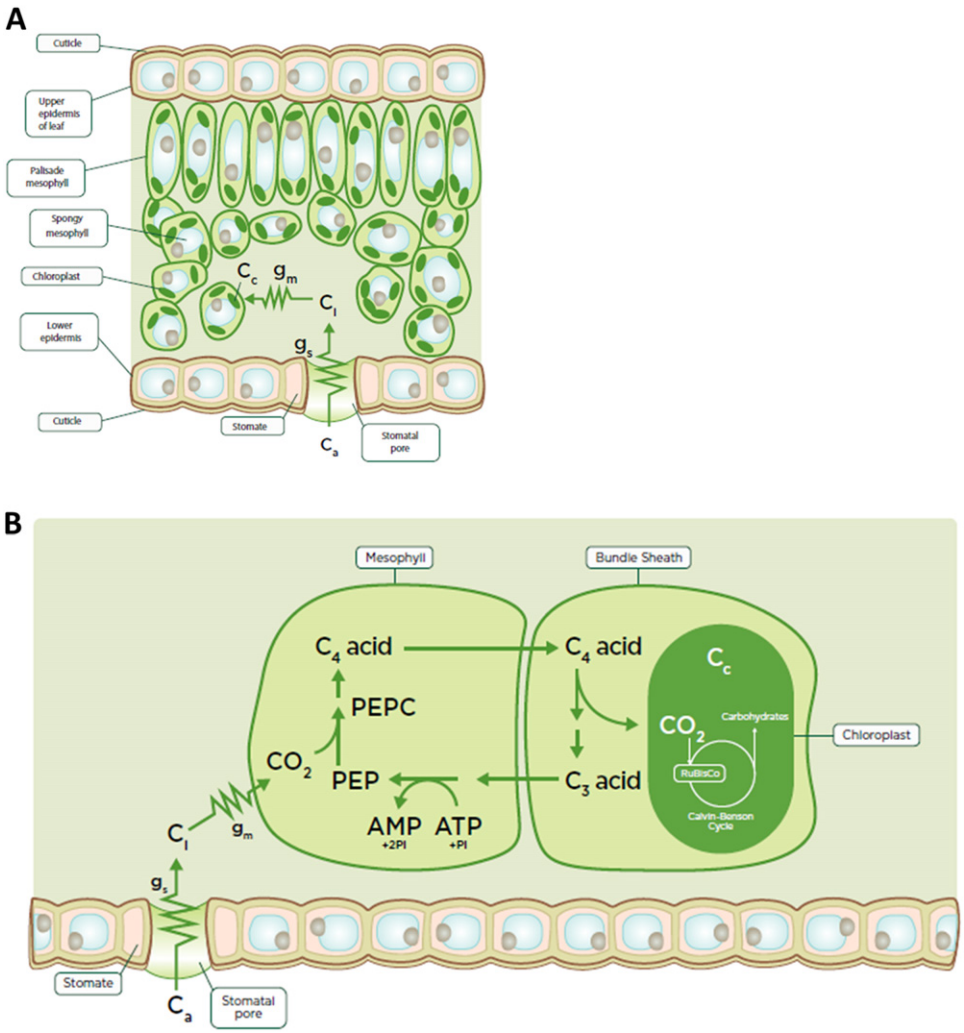

Fig. 3 CO_2 pathway from the atmosphere to the site of Rubisco. A. In a C3 leaf, CO_2 diffuses from high concentration in the atmosphere (C_a) to relatively lower concentration in the intercellular spaces of the leaf (C_i). The rate of diffusion between the atmosphere and the intercellular space (g_s) is governed by stomatal aperture, which is controlled by guard cells in the leaf epidermis. CO_2 in the intercellular space then diffuses through the apoplast/cell wall, plasma membrane, cytoplasm, chloroplast membrane, and stroma of mesophyll cells to reach the site of Rubisco. The CO_2 concentration in the chloroplast is termed C_c. Mesophyll conductance (g_m) governs the rate of diffusion between the intercellular space and the chloroplast. B. In a C4 leaf, initial CO_2 fixation occurs in the mesophyll cell, after which CO_2 is transported as a four-carbon molecule into the bundle sheath cell to the site of Rubisco.

the intercellular space within the leaf, where the concentration of CO_2 (C_i) has been drawn down by photosynthesis. The waxy cuticle of the leaf epidermis largely prevents gas exchange between the leaf and atmosphere. Thus, gas diffusion in and out of the leaf is controlled by stomata, or pores, in the leaf epidermis. Stomata in higher plants are encompassed by guard cells that open when a signal, such as light, triggers the influx of solutes, and therefore water, into the specialized epidermal cells, causing them to swell and therefore widen the pore to allow CO_2 into the leaf and in consequence the escape of water vapor from the leaf. The opposite process occurs when the stomata close (Lawson and Matthews, 2020). The rate at which CO_2 enters the intercellular space of the leaf from the atmosphere can be determined by Fick's law of diffusion

$$F_{gas} = \Delta C_{gas}/r$$

where F_{gas} represents the flux or net movement of a gas, ΔC_{gas} is the concentration gradient of a gas, and r represents the resistance to the movement of the gas. Here, ΔC_{gas} is determined as $C_a - C_i$, whereas r is governed by stomatal aperture which in turn determines conductance (g_s). From the intercellular space, CO_2 must diffuse through the apoplast/cell wall, plasma membrane, cytoplasm, chloroplast membrane and stroma of mesophyll cells to reach the site of CO_2 fixation within the chloroplast, where the concentration of CO_2 (C_c) is even lower than C_i. Thus, the rate of this passage is determined by C_i-C_c and the resistance to diffusion, which is governed by mesophyll conductance (g_m). Thus, both g_s and g_m contribute to determining C_c in C3 plants. In C4 plants, g_s and g_m determine the concentration of CO_2 in the mesophyll cell where CO_2 is initially fixed by PEPC to a

three-carbon molecule to form a four-carbon acid. The four-carbon acid is then transported to bundle sheath cells where it is decarboxylated to increase the concentration of CO_2 at the site of Rubisco (**Fig. 3(B)**). The pumping of CO_2 from mesophyll to bundle sheath cells requires additional energy in the form of ATP, but under most conditions the cost is offset by the benefits of increased C_c at Rubisco suppressing the Rubisco-catalyzed oxygenation reaction (see photorespiration). While C_c is a more accurate representation of CO_2 at the site of Rubisco than C_i, C_c is more difficult and less accurate to measure or estimate; therefore, C_i is often used instead of C_c to model CO_2 supply to Rubisco.

When stomata open to allow CO_2 diffusion into the leaf, water diffuses down its concentration gradient out of the leaf. Therefore, the leaf must tightly regulate stomatal opening/closing to both provide sufficient CO_2 for photosynthesis while limiting water loss. Due to the carbon-concentrating nature of C4 plants, leaf C_i/C_a is generally 0.4–0.6 (as opposed to 0.7–0.8 in C3 plants), which creates a large gradient in CO_2 concentration between the inner leaf and the outside air and thus a stronger driving force for CO_2 influx through stomata. Therefore, g_s can remain lower in C4 plants while maintaining an adequate CO_2 supply for photosynthesis, resulting in higher water use efficiency, or carbon gain per water loss. However, this balancing act in C3 plants is a major limitation to CO_2 supply for carboxylation. The regulation is even more difficult for plants grown in the field as light levels can fluctuate rapidly due to shading from diurnal changes in sun angle, wind movement of overhead foliage, and intermittent clouds (Slattery *et al.*, 2018). Slow stomatal opening when a leaf experiences a sudden increase in light intensity limits CO_2 supply for photosynthesis, while slow stomatal closure upon a decrease in light results in unnecessary water loss. Thus, faster stomatal responses to changes in light intensity could enhance photosynthesis while preventing wasteful water loss.

Certain stomatal guard cell traits are associated with more rapid stomatal movements but may vary by species and growth conditions (Lawson and Vialet-Chabrand, 2019). For example, smaller stomata seem to respond more rapidly when comparing closely related plants within the same species or genus. However, such relationships do not hold across broader comparisons. Other anatomical considerations include stomata type/shape, as dumbbell-shaped stomata, as are found in grass species, respond more rapidly than elliptical-shaped stomata in dicot crops. Flexibility of subsidiary cells, which border guard cells, can enhance stomatal movements by transferring turgor to guard cells, thereby allowing more space for guard cell expansion. Additional influences on stomatal response kinetics may stem from structural properties of guard cell walls, whereas biochemical considerations, such as the speed of solute transport between subsidiary cells and guard cells, also affect the speed of stomatal opening or closure and may hold opportunities for improving stomatal movement kinetics in fluctuating environments. Recent progress in engineering for more rapid stomata has been achieved through introduction of a synthetic K^+ channel in the guard cells of the model plant Arabidopsis through increasing the rate of solute influx into the guard cell (Papanatsiou *et al.*, 2019). In terms of growth conditions, well-watered environments allow some plants to maintain a higher g_s when light levels are low, thus allowing g_s to reach levels that support maximal photosynthetic rates more rapidly upon exposure to high light. In addition, non-limiting water supply may lead to stomata continuing to open well past reaching the necessary CO_2 supply (C_i) for maximal rates of photosynthesis, which is termed "overshoot" (McAusland *et al.*, 2016) and may be beneficial for maintaining non-stressful leaf temperatures or for buffering photosynthesis in relatively more dynamic light environments but may deplete soil moisture more rapidly, sacrificing soil water reserves. There may also be costs to more rapid stomatal movements in terms of energy and solutes compared to what is gained in photosynthesis or water use efficiency in a dynamic environment. In addition, faster stomatal kinetics may not be sustainable after a few cycles of opening/closing. Thus, a balance is required to optimize stomatal movements, photosynthesis, and water use efficiency, depending on growth conditions.

Less is known about g_m, the mechanism(s) controlling g_m, and its limitations. Aquaporin levels, carbonic anhydrase concentration, and leaf and cell anatomy likely influence g_m (Flexas *et al.*, 2012), and g_m may present greater limitations to photosynthesis as temperatures increase (Evans and von Caemmerer, 2013) and in response to fluctuating light (Huang *et al.*, 2015; Vialet-Chabrand *et al.*, 2017). Thus a greater understanding of g_m mechanisms and more strategies for g_m improvement are needed to minimize CO_2 supply limitation to photosynthesis.

Carbon Assimilation

Better Rubisco

Rubisco catalyzes the carboxylation of the 5-carbon sugar RuBP to ultimately yield the net production of two molecules of glycerate 3-phosphate (G3P), which leaves use to produce carbohydrates that are the building blocks for the vast array of molecules and compounds that plants make. As the most abundant protein in the biosphere (Bar-On and Milo, 2019), the inefficiency of Rubisco represents a major limitation to photosynthesis. Not only is the enzyme catalytically slow, it also has a relatively low specificity for CO_2 versus O_2, likely a consequence of having evolved when atmospheric CO_2 levels were high and O_2 levels were low or absent. This presages the oxygenation of RuBP, which then requires the energetically costly photorespiratory pathway to recycle the inhibitory byproducts (see below). While there is genetic variability in Rubisco specificity there is seemingly an inherent tradeoff between Rubisco catalytic rate and specificity. Nevertheless, genetic diversity in some of the world's major crops and their wild relatives may provide new materials for improving Rubisco kinetics and specificity (Orr *et al.*, 2016), which will be even more important as temperatures rise and the specificity for CO_2 over O_2 in solution declines and the solubility of CO_2 declines to a greater degree than the solubility of O_2. However, there has so far been only limited success in transplanting foreign Rubisco into different species to take advantage of this genetic diversity. But the expression of Rubisco genes in the chloroplast genome as well as co-transformation with Rubisco chaperone proteins holds promise (Bracher *et al.*, 2017; Conlan *et al.*, 2019), as

does the long-sought after complete expression and assembly of functional higher plant Rubisco in *E. coli* (Aigner *et al.*, 2017). Efforts to re-engineer Rubisco for improved kinetic properties have to date been unsuccessful even with the guidance of a high-resolution atomic structure of the Rubisco enzyme complex.

Improved Rubisco activase

Before Rubisco can catalyze carboxylation of RuBP, the enzyme must first be activated. Activation occurs through the carbamylation of a lysine residue in the catalytic site of Rubisco, which is then stabilized by Mg^{2+} binding. However, sugar phosphates, such as D-xylulose-1,5-bisphosphate (XuBP) or even Rubisco's substrate RuBP, can bind to the catalytic site of the non-activated enzyme, thereby blocking activation. In addition, XuBP and other sugar phosphates, such as 2-carboxy-D-arabinitol 1-phosphate (CA1P), D-glycero-2,3-pentodiulose-1,5-bisphosphate (PDBP), 2-carboxytetritol-1,4-bisphosphate (CTBP), and 3-ketoarabinitol-1,5-bisphosphate (KABP), can bind Rubisco after activation, thereby inhibiting catalysis of the activated enzyme. Rubisco activase (Rca) is required to sustain the activated state of Rubisco by facilitating the removal of these inhibitors from its catalytic site (Wang and Portis, 1992; Portis, 2003). Although levels of Rca are not normally limiting in steady-state conditions, higher levels are sometimes associated with increased yield (Yin *et al.*, 2014). In addition, higher levels are also beneficial for more rapid induction of photosynthesis in fluctuating light environments (Yamori *et al.*, 2012). However, if higher levels of Rca occur at the expense of Rubisco levels, this can be detrimental to photosynthesis in steady-state conditions (Fukayama *et al.*, 2012). Thus, fine-tuning the regulation of Rca activation may offer a solution for more rapid activation of Rubisco upon low-to-high light transitions that is predicted to significantly increase diurnal canopy carbon gain (Carmo-Silva *et al.*, 2015). To realize this potential, the sensitivity of Rca to high temperatures (Feller *et al.*, 1998) will necessitate greater thermotolerance to maintain photosynthetic rates in warming climates.

CO_2 concentrating mechanisms

As noted above, Rubisco often catalyzes the oxygenation, rather than the carboxylation, of RuBP, and rising temperatures will act to increase the ratio of oxygenation to carboxylation. However, improving the specificity of Rubisco while maintaining or enhancing the speed of the enzyme has not yet been achieved. Thus, concentrating CO_2 at the site of Rubisco could help prevent or at least lower the rate of the oxygenation reaction and may be possible through several approaches. One approach is through converting C3 photosynthesis to C4 photosynthesis, which has the added benefits of improving water and nitrogen use efficiencies. Although C4 photosynthesis coevolved many times in different plant lineages, this approach requires complex alterations to leaf anatomy and the tissue-specific expression of several enzymes. The ongoing project of converting C3 rice to C4 rice has made considerable progress in the last 10 years through identifying the genes of the major enzymes and transporters involved in the C4 pathway. However, precisely regulating the expression of these genes within rice poses an additional hurdle, as does altering rice leaf anatomy to complete the complex transformation (Ermakova *et al.*, 2020). An intermediate step between C3 and C4 photosynthesis involves concentrating CO_2 through a photorespiratory CO_2 pump, whereby CO_2 released through photorespiration is shuttled via the two-carbon molecule glycine (thus given the name "C2 photosynthesis") to the bundle sheath. Plants with this photorespiratory pump are often referred to as C3-C4 intermediates and may have C_c levels up to three times higher than in their C3 counterparts (Keerberg *et al.*, 2014). Thus, the introduction of this pump into C3 plants could serve as an intermediate step to full integration of C4 photosynthesis for improving photosynthetic efficiency. Another approach is through the introduction of cyanobacterial carboxysomes and associated enzymes into plant chloroplasts. This involves engineering plants to produce carboxysomes within the chloroplast while at the same time concentrating bicarbonate in the chloroplast and isolating the conversion of bicarbonate to CO_2 solely within the carboxysome. Current progress in introducing carboxysomes into chloroplasts has succeeded in assembling carboxysome structures within the chloroplast that contain cyanobacterial Rubisco (Lin *et al.*, 2014; Long *et al.*, 2018). While less is known about pyrenoids, which are present in many types of algae and are the location of approximately one-third of global CO_2 fixation, introduction of these carbon concentrating compartments within the chloroplast represents a similar strategy. Recent identification of transport proteins required to transport bicarbonate into these types of structures has provided a crucial step in being able to engineer these structures into plants with the appropriate accompanying enzymes (Mukherjee *et al.*, 2019).

More efficient photorespiratory pathways

As noted above, Rubisco catalyzes the oxygenation of RuBP, which results in the formation of one molecule of G3P and one molecule of 2-phosphoglycolate (2PG), the latter of which is inhibitory to several photosynthetic enzymes (Flügel *et al.*, 2017) and requires recycling through the photorespiratory pathway. 2PG is first converted to glycolate, which is then transported out of the chloroplast and into the peroxisome where it is converted to glycine before it exits the peroxisome and enters the mitochondria. There, glycine undergoes conversion to serine, during which ammonium and CO_2 are released. Serine then exits the mitochondria and enters the peroxisome again to be converted to glycerate, which is transported into the chloroplast and reenters the Calvin-Benson cycle as 3-phosphoglycerate (3PG). Thus, the native pathway requires multiple steps that span the peroxisome and mitochondria and results in energy usage and loss of previously fixed CO_2 and ammonium, which both require additional energy for refixation. Each Rubisco oxygenation costs 3.5 ATP and 2 NADH equivalents (Walker *et al.*, 2016). The energy cost for a C3 leaf in a crop canopy has been estimated as 32% of ATP and 28% of NADPH produced under growth conditions of current atmospheric CO_2 levels and 25°C. Therefore, photorespiration represents a major inefficiency to C3 photosynthesis (Walker *et al.*, 2016).

While concentrating CO_2 at Rubisco will help limit oxygenation reactions, photorespiration will still likely occur, especially as temperatures rise and Rubisco's specificity for CO_2 versus O_2 declines and the solubility of CO_2 declines more rapidly than that of O_2. Thus, there are benefits to reducing the costs of photorespiration, which can potentially be achieved in several ways. One possibility involves engineering a more rapid recycling through the native pathway by increased expression of potentially rate-limiting enzymes. For example, overexpressing the protein subunits of glycolate dehydrogenase, which plays a key role in glycine conversion to serine in the mitochondria, results in increased plant biomass (López-Calcagno *et al.*, 2019). In addition, overexpressing the enzyme responsible for 2PG degradation, 2PG phosphatase, increases photosynthetic rates under abiotic stress conditions (Timm *et al.*, 2019). A second strategy involves introduction of more efficient synthetic pathways. To date, two synthetic pathways have been used to bypass several steps of the native pathway while containing all reactions within the chloroplast (Kebeish *et al.*, 2007; Maier *et al.*, 2012). These strategies result in greater biomass accumulation in field conditions, showing promise for improving crop yields (Shen *et al.*, 2019; South *et al.*, 2019). Additionally, entirely new pathways could be used to facilitate more productive conversion of 2PG to other substrates within the Calvin-Benson cycle, such as a *de novo* pathway contained in the chloroplast that ultimately converts 2PG to RuBP rather than re-entrance into the Calvin-Benson cycle at the 3PG step, thus also bypassing most of the steps involved in RuBP regeneration (Ort *et al.*, 2015; Bar-Even, 2018; Weber and Bar-Even, 2019 and see below).

RuBP Regeneration

Electron Transport Chain

As C_i increases in a C3 leaf, leaf photosynthesis reaches the inflection point where RuBP regeneration becomes more limiting than CO_2 supply to photosynthesis. Therefore, as C_a and temperature increase due to anthropogenic causes, RuBP regeneration, which is powered by ATP and NADPH formed due to the electron transport chain in the thylakoid membrane, will more frequently limit photosynthesis. ATP and NADPH are formed through the light reactions in the thylakoid lumen. In C3 plants, the linear flow of electrons through the electron transport chain is localized to mesophyll cells. There, light energy absorbed by the chlorophylls in the light harvesting complex of photosystem II (PSII) is first funneled to the reaction center, which in turn results in the splitting of H_2O. Protons from H_2O are deposited in the lumen while an electron is transferred to plastoquinone (PQ) to form PQH_2 on the opposite side of the thylakoid membrane, which results in proton uptake from the stroma. The electrons are then transferred from PSII to cytochrome b_6f (Cyt b_6f) by PQH_2 resulting in additional proton deposition in the thylakoid lumen. Electrons then flow from Cyt b_6f to photosystem I (PSI) via plastocyanin (PC). Electrons are then directly transferred to ferredoxin (Fd), followed by ferredoxin-NADP+ reductase (FNR) to reduce NADP+ to NADPH, again involving proton uptake from the stroma. Meanwhile, proton accumulation in the lumen results in a gradient in both electrical charge and concentration across the thylakoid membrane, thereby creating a PMF that drives the formation of ATP via proton flow through ATP synthase. The electron transport chain in C4 mesophyll cells functions similarly as in C3 mesophyll cells. In bundle sheath cells, however, there is a greater need for ATP. Thus, there is little to no PSII, and electron transport primarily flows between Cyt b_6f, PSI and predominantly NADPH dehydrogenase (NDH) through what is termed cyclic electron flow (CEF) (Majeran *et al.*, 2008). As a result, a PMF is formed, which leads to ATP production but not NADP reduction. The inefficiencies of the electron transport chain as well as limiting enzymes within the regeneration reactions are discussed below.

Broader light absorption

As noted above, plant photosynthesis (i.e., water-oxidizing/oxygenic photosynthesis) uses light in the 400–700 nm range, which corresponds to visible light and less than half the available energy from the sun. The major light absorbing pigments in plants are chlorophylls *a* and *b* (**Fig. 4**). However, some photosynthetic bacteria are able to use light outside of this range (near-infrared), which presents an opportunity for more light capture. For example, some purple bacteria type 2 reaction centers use bacteriochlorophyll *b*, which absorbs wavelengths beyond 1000 nm. Some oxygen-evolving cyanobacteria, such as *Acaryochloris marina*, use chlorophyll *d*, which absorbs wavelengths up to 730 nm, making approximately 30% more photons available to drive photosynthesis. *Chroococcidiopsis thermalis* supplements chlorophyll *a* with chlorophyll *f* when grown under far-red light, allowing a red absorption maximum at >760 nm (**Fig. 4**).

In addition to using the same chlorophylls and therefore competing for similar wavelengths of light for oxygenic photosynthesis, PSII and PSI reaction centers also operate in series, thereby presenting a major inefficiency to electron transport in photosynthesis. Since bacterial reaction centers use different chlorophylls that absorb wavelengths much further into the infrared (**Fig. 4**), replacing PSI with those from bacteria, or at least replacing the pigments within the reaction centers with those from bacteria, could circumvent this issue of spectral overlap of the two photosystems. Thus, the photosystems would not compete for the same wavelengths and would essentially double the light use efficiency of two-photosystem photosynthesis (Blankenship *et al.*, 2011).

Limiting enzymes of electron transport and RuBP regeneration

Rate-limiting enzymes involved with electron transport slow the production of ATP and NADPH, which can limit RuBP availability for carboxylation reactions. Cyt b_6f represents a key control point within both linear and cyclic electron transport. Therefore, levels of Cyt b_6f could limit electron transport in both C3 and C4 plants under high light. Increasing the expression of Cyt b_6f through overexpression of the Rieske FeS protein within Cyt b_6f leads to assembly of higher levels of Cyt b_6f in the thylakoid membrane and increased light conversion efficiency, higher PMF, and higher leaf photosynthesis (Simkin *et al.*, 2017b; Ermakova *et al.*, 2019), thereby providing a target for improving the efficiency of electron transport and RuBP regeneration.

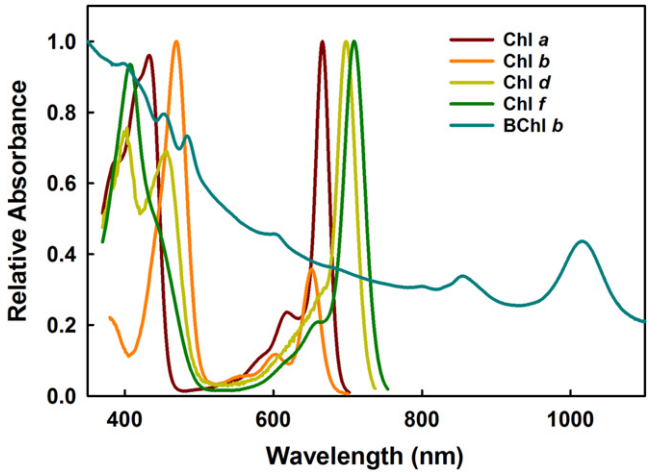

Fig. 4 Relative absorbance spectra of photosynthetic pigments. Chlorophylls (Chl) *a* and *b* are used by photosystems II and I in higher plants, whereas Chl *d* is used by the oxygen-evolving cyanobacteria *Acaryochloris marina*, and Chl *f* is found in *Chroococcidiopsis thermalis*. Bacteriochlorophyll (BChl) *b* is present in the type 2 reaction centers of purple bacteria. Chl absorbance was measured in methanol, and BChl absorbance was measured *in vivo*. Data were obtained from http://vplapps.astro.washington.edu/pigments.

In addition to more efficient energy capture and production of ATP and NADPH, previous leaf photosynthesis modeling has demonstrated some enzymes in the RuBP regeneration cycle may limit the regeneration rate at current atmospheric CO_2 levels (Zhu *et al.*, 2007). Most notable of these is sedoheptulose-1,7-bisphosphatase (SBPase). Increased expression of SBPase has shown promise in increasing RuBP regeneration rates, both singly and in combination with fructose-1,6-bisphosphatase (FBPase), especially under future climate conditions simulated in the field, such as elevated CO_2 and higher temperatures (Rosenthal *et al.*, 2011; Köhler *et al.*, 2017). Fructose-1,6-bisphosphate aldolase (FBPA) overexpression alone and in combination with SBPase also increases photosynthetic efficiency and biomass production in the model plant Arabidopsis, and the effect is even greater in combination with overexpression of the H-protein of glycolate dehydrogenase involved in the photorespiratory pathway (Simkin *et al.*, 2017a).

Although other enzymes are highly regulated in the Calvin-Benson cycle, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK), altered expression of these enzymes does not lead to increased photosynthetic efficiency (Price *et al.*, 1995; Paul *et al.*, 1995). However, induction of the activity of these light-activated enzymes can take minutes upon low-to-high light transitions (Sassenrath-Cole and Pearcy, 1994; Sassenrath-Cole *et al.*, 1994). Thus, their activation can be limiting to photosynthesis in fluctuating light environments. Thioredoxin proteins, including the *f* and *m* types, activate these enzymes, and *m*-type thioredoxins have been shown to affect photosynthetic efficiency when light is fluctuating more so than in steady-state conditions (Thormählen *et al.*, 2016). The NADPH-thioredoxin reductase C (NTRC) pathway, which affects photosynthetic efficiency through maintaining the NADPH redox status of the stroma, also cooperates with the ferredoxin-thioredoxin system to regulate FBPase and SBPase activity. PRK and GAPDH are also redox-regulated, but they are fully activated after dissociation from the complex they form with a protein named CP12, which occurs more rapidly than the redox regulation and allows for faster induction (Howard *et al.*, 2008; Marri *et al.*, 2009). As these mechanisms become clearer, modification may be possible to increase the speed of enzyme induction for more efficient photosynthesis in fluctuating light.

Wasting Less Light: Leaf and Canopy

Enhancing responsiveness of photoprotective mechanisms

Light fluctuations pose a challenge for leaves to use light efficiently. When light increases above saturation, the level of which can decrease due to additional stresses, leaves employ the photoprotective mechanism NPQ in the photosystem II antenna to dissipate excess light as heat to prevent damage to PSII and associated photosynthetic machinery (see review by Ruban (2016)). This process, while protecting the leaf, represents an inefficiency when it remains deployed during a sudden decrease in light (**Fig. 2**), as frequently happens in plant canopies such as when wind changes the shading of a lower leaf by higher leaves in the canopy or as the sun leaf angle changes. Although the induction of this photoprotective process occurs within seconds, the relaxation rate is relatively slower, occurring on the time scale of minutes and longer. During this time after a high to low light transition, light that could be used for photosynthesis is instead dissipated as heat, which has been estimated to limit daily canopy carbon assimilation of crops in the field by as much as ~30% (Zhu *et al.*, 2004b). Thus, a more rapid relaxation of NPQ over the course of a day could potentially markedly increase overall carbon assimilation by a canopy. Although there are several NPQ components required for protecting photosynthetic machinery, energy-dependent quenching (qE) is the most rapid, occurring on the scale of seconds to minutes. Upon exposure to high light, qE induction depends on acidification of the lumen, which is sensed by PsbS, a protein associated with PSII, and this signals LHCII to undergo a conformation change. At the same time, violaxanthin converts to zeaxanthin via violaxanthin deepoxidase through the xanthophyll cycle. The reverse reaction is catalyzed by zeaxanthin epoxidase

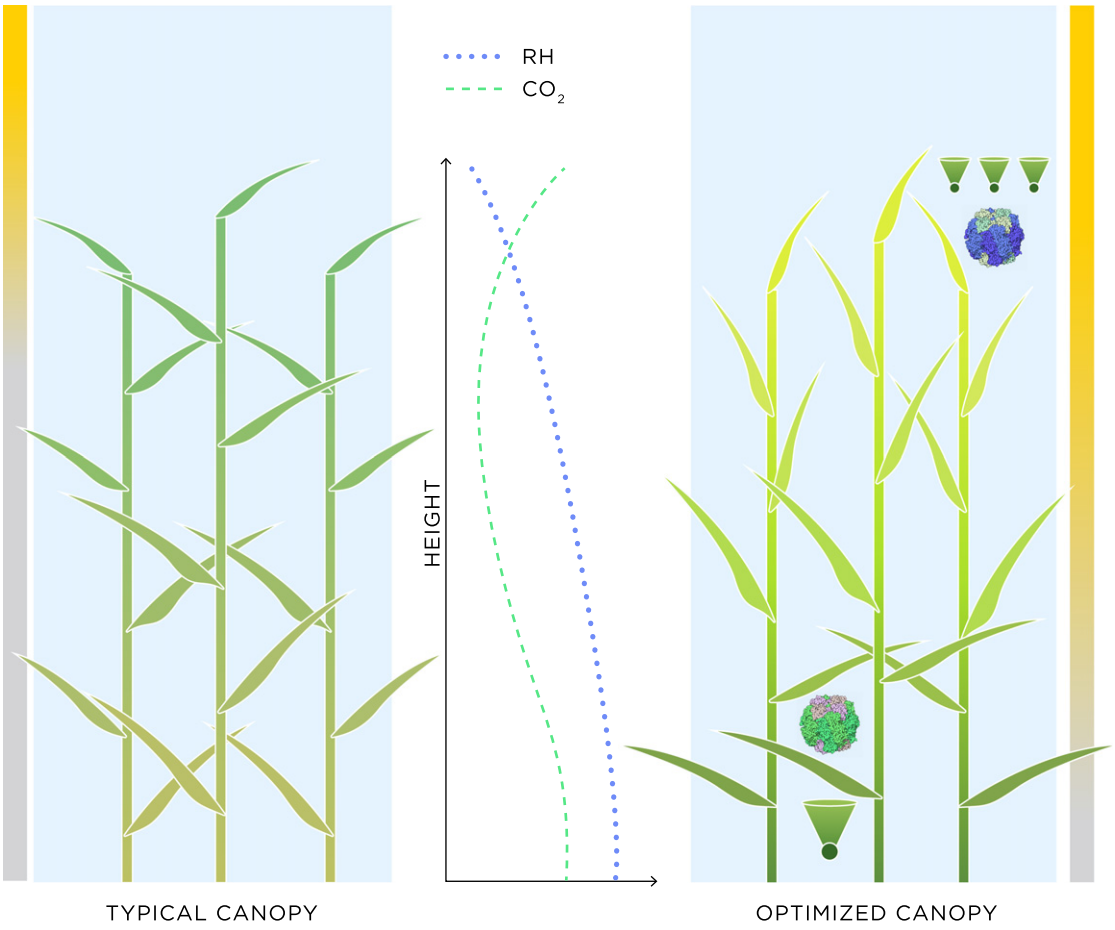

Fig. 5 Optimizing the distribution of photosynthetic machinery within a canopy. In a typical canopy (left), horizontal dark green leaves at the top of the canopy absorb most of the incident sunlight (represented by yellow vertical bar) and very little penetrates to the bottom of the canopy. In an optimized canopy (right), more vertically-oriented light-green leaves at the top of the canopy allow a more even distribution of sunlight in the canopy, which allows more photosynthesis to occur in the lower canopy where relative humidity (RH) and CO_2 are higher. Further optimization results from engineering for a Rubisco (protein diagram) with a higher catalytic rate at the top of the canopy and higher specificity lower in the canopy. An optimized canopy would also have fewer antennae pigments per reaction center (cones) at the top with larger antennae serving fewer reaction centers at the bottom. Modified from Ort, D.R., Merchant, S.S., Alric, J., *et al.*, 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proceedings of the National Academy of Sciences 112, 8529–8536.

upon exposure to low light. Singular overexpression of these individual enzymes results in negative side effects. For example, overexpression of PsbS increases induction and relaxation rates and overall qE capacity, but the relaxed levels of qE are still higher than needed under non-stressful light conditions (Hubbart *et al.*, 2012). However, simultaneous overexpression of PsbS, violaxanthin deepoxidase, and zeaxanthin epoxidase increases NPQ kinetics while maintaining proper levels of qE. This has been shown to increase biomass by 15% in field-grown plants and thus represents a strategy for substantial improvements to photosynthetic efficiency under fluctuating light (Kromdijk *et al.*, 2016).

The photoinhibition of PSI has received relatively less attention than that of PSII. PSI is relatively insensitive to excess light energy and can dissipate excessive excitation energy through the water-water cycle and direct quenching by $P700^+$. However, excess electrons received from PSII through the electron transport chain can cause photoinhibition of PSI, which requires more time to repair than PSII. This often occurs in fluctuating light conditions, particularly during low to high light transitions when NPQ of PSII has not yet fully engaged (Huang *et al.*, 2019). Thus, more rapid induction of NPQ and stronger electron sinks on the acceptor side of PSI may be needed to avoid PSI photoinhibition and the associated negative impacts on carbon assimilation and photosynthetic efficiency.

Light distribution

Why engineer plants with higher light absorption if leaf photosynthesis currently saturates at less than half the light levels received from the sun on a clear sunny day? Although a leaf at the top of a crop canopy is exposed to light levels well above what is needed to reach maximum levels of leaf photosynthesis, leaves lower in the canopy are most often operating under light limitation due to over absorption at the top of the canopy (**Fig. 5**). This non-optimal light distribution perhaps could be addressed by lowering leaf chlorophyll concentrations in the upper leaves of crop canopies. High chlorophyll concentration is a very good competitive

strategy in a natural setting by both ensuring sufficient light absorption for an individual plant while preventing light from reaching surrounding competitors, thus leading to greater biological fitness. This likely led to the current high chlorophyll concentrations seen in today's crops. However, this evolutionary strategy probably is not ideal and lowers overall carbon gain by crop canopies. Indeed, modeling shows chlorophyll content in a crop canopy can be dramatically decreased without a canopy carbon gain penalty (Walker et al., 2018), and reinvesting the nitrogen that would otherwise be used for chlorophyll biosynthesis elsewhere in the photosynthetic machinery could improve canopy carbon assimilation (Zhu *et al.*, 2007; Song *et al.*, 2017).

Optimizing light distribution within the monocultures typically seen in food and feed production might be achieved through simultaneously altering leaf pigmentation and leaf orientation. Light-green leaves oriented in an upright position at the top of the canopy would absorb less light, allowing more light to penetrate deeper in the canopy where dark-green horizontal leaves would absorb the remaining light (**Fig. 5**). Although lowering absorption of crop leaves results in light transmitted to the soil early in canopy development and increased reflectance from the top of the canopy, careful timing of pigment reduction (i.e., employing a "switch" to reduce chlorophyll biosynthesis in developing leaves once the canopy has completely closed) could lessen these impacts. A deeper light distribution within a canopy would be predicted to have other benefits, as humidity is often greater lower in canopies (Drewry *et al.*, 2014); thus stomata could remain open for increased CO_2 supply without the same rates of water loss seen at the top of the canopy, thereby improving canopy carbon gain and water use efficiency. Other aspects of photosynthesis could also be optimized with canopy depth, such as the type of Rubisco present. A Rubisco with a higher catalytic rate even with the sacrifice in specificity factor would be beneficial in the upper canopy where light energy is more available, whereas higher specificity for CO_2 would be more beneficial in the lower canopy to minimize light energy used in photorespiration (Zhu *et al.*, 2004a). In addition, a higher number of photosystems with smaller antennae in the upper canopy and a lower number of photosystems in the lower canopy with larger antennae would also ensure more efficient light use throughout the canopy.

Triose Phosphate Utilization (TPU)

Carbon Sink Limitation

G3P formed in the Calvin-Benson cycle is reduced to triose phosphates, which are then primarily used to form sucrose and starch. However, triose phosphates from the Calvin-Benson cycle are also used in other pathways, such as the synthesis of aromatic amino acids, branched-chain amino acids, fatty acids, and isoprenoids. The synthesis of these compounds releases phosphate, which returns to the chloroplast and is used during the conversion of ADP to ATP by the ATP synthase in the thylakoid membrane. Low rates of TPU can lead to low phosphate availability and consequently slower ATP formation; therefore, the ratio of ATP to ADP decreases, which leads to a negative feedback on photosynthetic electron transport and carbon fixation. The resulting plateau or decline in photosynthesis is visible from C3 photosynthetic CO_2-response curves at high C_i where carboxylation and electron transport no longer limit photosynthetic rates (**Fig. 2B**; McClain and Sharkey, 2019). This is not evident in the C4 photosynthetic CO_2-response curve, however, as gas exchange techniques do not capture TPU at high CO_2 levels due to the nature of the C4 CO_2 concentrating pump.

It is unclear how future climate conditions will impact the limitation of TPU on photosynthesis. Since TPU limits C3 photosynthesis to the greatest extent at higher C_i where carboxylation and electron transport no longer limit photosynthesis, TPU limitation to photosynthesis may become more limiting as CO_2 levels rise. TPU is also temperature sensitive due to the sensitivity of several enzymes involved in TPU, such as sucrose-phosphate synthase, and may be the most temperature-sensitive limitation to photosynthesis. However, TPU is generally more limiting at lower temperatures and thus may be less limiting to photosynthesis with higher growth temperatures predicted in future climates (McClain and Sharkey, 2019). Additional studies are necessary to determine whether TPU limitation to photosynthesis will increase or decrease in future climate conditions.

Summary

New and sustainable methods of improving crop productivity are required to feed and fuel the growing global human population, especially when accounting for the threat of global climate change on crop yields. Improving the efficiency of photosynthesis through implementing the strategies discussed above, both through exploiting natural genetic variation and bioengineering, presents an opportunity to do so. Additional strategies will likely arise as research continues in this field. All of these may require concurrent efforts, such as increasing grain sink strength, to ensure the additional carbon assimilated through higher photosynthetic rates is translated into harvestable yield.

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