Dear Prof. Helariutta, Prof. Krieger-Liszkay and Prof. Morosinotto,

We would like to submit our mini-review manuscript, *New Avenues in Photosynthesis: From Light Harvesting to Global Modeling*, by Tiina Tosens, Alessandro Alboresi, Herbert van Amerongen, Roberto Bassi, Florian A. Busch, Giovanni Consoli, Oliver Ebenhöh, Jaume Flexas, Jeremy Harbinson, Peter Jahns, Nina Kamennaya, David M. Kramer, Johannes Kromdijk, Tracy Lawson, Erik Murchie, Ülo Niinemets, Sara Natale, Dennis J. Nürnberg, Andrea Persello, Paolo Pesaresi, Christine Raines, Urte Schlüter, Tom P. J. M. Theewuenen, Stefan Timm, Dimitri Tolleter, and Andreas P. M. Weber, for consideration in the Special Issue on *European Photosynthesis Congress* in *Physiologia Plantarum,* with Prof. Tomas Morosinotto as preferred editor.

This review synthesizes selected recent advancements presented at the *Second European Congress of Photosynthesis Research*, with a particular focus on improving photosynthetic efficiency and advancing photosynthesis modeling across the scales. The discussed topics directly contribute to addressing critical global challenges, including food security and environmental sustainability.

We confirm that this manuscript has not been submitted or published elsewhere, and all authors have approved its submission to *Physiologia Plantarum*. Additionally, we declare that there are no competing interests related to this work.

Thank you for your time and consideration,

With kind regards,

Prof. Tiina Tosens

Chair of Crop Science and Plant Biology

Estonian University of Life Sciences,

D190a, Kreutzwaldi 5

51014 Tartu Estonia

New avenues in photosynthesis: from light harvesting to global modeling

Tiina Tosens[1], Alessandro Alboresi[2], Herbert van Amerongen[3], Roberto Bassi[4], Florian A. Busch[5], Giovanni Consoli[6], Oliver Ebenhöh[7], Jaume Flexas[8], Jeremy Harbinson[3], Peter Jahns[7], Nina Kamennaya[9], David M Kramer[11], Johannes Kromdijk[12], Tracy Lawson[10,13], Erik Murchie[14], Ülo Niinemets[1], Sara Natale[2], Dennis J. Nürnberg[15,16], Andrea Persello[17], Paolo Pesaresi[17], Christine Raines[12], Urte Schlüter[7], Tom P. J. M. Theewunen[18], Stefan Timm[19], Dimitri Tolleter[20], Andreas P.M Weber[7]

1. Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 51006 Tartu, Estonia
2. Department of Biology, University of Padova, 35131 Padova, Italy
3. Laboratory of Biophysics, Wageningen University & Research, Wageningen 6708 WE, The Netherlands
4. Dipartimento di Biotecnologie, Università di Verona, Strada Le Grazie 15, 37134 Verona, Italy
5. School of Biosciences and Birmingham Institute of Forest Research, University of Birmingham, Birmingham, UK
6. Department of Life Sciences, Imperial College; London, SW7 2AZ, United Kingdom
7. Institute of Plant Biochemistry, Cluster of Excellence on Plant Science (CEPLAS), Heinrich Heine University, Universitätsstrasse 1, 40225 Düsseldorf, Germany
8. Research Group on Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears (UIB) - Institut d'Investigacions Agroambientals i d'Economia de l'Aigua (INAGEA), Ctra Valldemossa km 7.5, 07122 Palma, Illes Balears, Spain.
9. French Associates Institute for Agriculture and Biotechnology of Drylands, Blaustein Institutes for Desert Research & Goldman Sonnenfeldt School of Sustainability and Climate Change, Ben-Gurion University of the Negev, Israel
10. Carl R. Woese Institute for Genomic Biology, University of Illinois, 1206 West Gregory Drive, Urbana, IL 61801, USA.
11. Jan IngenHouz Institute, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands
12. Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, United Kingdom.
13. School of Life Sciences, University of Essex, Colchester, Essex CO4 3SQ, UK 13.

14. Carl R. Woese Institute for Genomic Biology, University of Illinois, 1206 West Gregory Drive, Urbana, IL 61801, USA.
15. Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK.
16. Institute of Experimental Physics, Freie Universität Berlin, Berlin, Germany
17. Department of Biosciences, University of Milan, 20133, Milan, Italy
18. Laboratory of Genetics, Wageningen University & Research, Wageningen 6708 PB, The Netherlands
19. Department of Plant Physiology, Institute for Biological Sciences, University of Rostock, Rostock, Germany
20. Interdisciplinary Research Institute of Grenoble, IRIG-LPCV, Grenoble Alpes University, CNRS, CEA, INRAE, 38000 Grenoble, France

Abstract

Photosynthesis is the foundation of life on Earth, serving as the primary energy source while regulating global carbon and water cycles, thereby shaping climate and vegetation. Advancing photosynthesis research is essential for improving crop productivity and refining photosynthesis models across scales, addressing critical global challenges such as food security and environmental sustainability.

This mini-review synthesizes selection of recent advancements presented at the Second European Congress of Photosynthesis Research, with a particular focus on improving photosynthesis efficiency and photosynthesis modeling across the scales. We begin by exploring strategies to optimize light harvesting and carbon fixation to enhance photosynthesis and yield leading canopy level improvements. Alongside synthetic biology, we examine recent advancements in harnessing natural variability in key photosynthetic traits, considering both methodological innovations and the vast reservoir of opportunities they present. Additionally, we highlight unique insights gained from plants adapted to extreme environments, offering pathways to simultaneously improve photosynthetic efficiency and resilience. Throughout, we emphasize the importance of a holistic approach, integrating dynamic modeling of metabolic processes to bridge these advancements. We also discuss the recent advancements in mesophyll conductance research discussed in congress and reliable approaches of integrating in photosynthesis estimations across the scales for improved photosynthesis estimations.

Additionally, we discuss the new initiatives launched—such as an open science platform and a dedicated journal for plant ecophysiology. We conclude this minireview with a forward-looking outline, highlighting key next steps toward achieving meaningful improvements in photosynthesis, yield and resilience and modeling.

1 INTRODUCTION

The Second European Congress of Photosynthesis Research brought together over 300 scientists from diverse career stages to discuss the latest advancements in photosynthesis research. Set against the historic backdrop of the Orto Botanico di Padova—the world's oldest botanical garden, featuring the iconic Mediterranean dwarf palm (*Chamaerops humilis*), planted in 1585— at that time, atmospheric CO_2 concentration was around 280 μmol mol^{-1}; today, it is 420 μmol mol^{-1} and rising. This dramatic shift, alongside changes in other environmental drivers and human population, underscores the urgent need for innovation to address ongoing environmental challenges. Photosynthesis, underpinning plant productivity, lies at the heart of these challenges, with rapid CO_2 rise affecting primary productivity in wild and cultivated plants.

Responding to these challenges, the ongoing research in photosynthesis has adopted a multifaceted approach, focusing on improving photosynthesis at leaf and canopy levels. Global food demand is projected to increase 50%–100% by 2050 (van Dijk et al., 2021). Carbon assimilation and plant growth continue to rise with increasing CO_2 availability (Smith et al., 2023), suggesting further gains may still be possible. Since photosynthesis directly affects crop yields (Smith et al., 2023), optimizing photosynthetic efficiency at both leaf and canopy levels has become central to increase crop productivity. In addition to synthetic biology, leveraging natural variability through modern genetics—by tapping into the vast pool of natural traits and their combinations—is an emerging trend that provides alternative strategies for enhancing photosynthesis and resilience (Croce et al., 2024). In this context, analyzing trade-offs in photosynthetic acclimation to various environmental conditions can reveal interesting trait combinations that simultaneously enhance both resilience and photosynthesis in crops.

Accurate modeling of photosynthesis across scales and its responses to environmental stresses is critical for predicting plant productivity, ecosystem dynamics, and climate change impacts and developing mitigation pathways (Masson-Delmotte et al., 2021). Current Earth System models, mainly resting on advancements of the Farquhar et al. (1980) model, have yet

to fully integrate recent physiological discoveries like mesophyll conductance, for improved simulations of photosynthetic sensitivity to environmental factors, especially CO_2 (see mechanisms discussed in Fig. 1).

Notably, the congress launched two transformative initiatives. David Kramer introduced an open research platform by The Jan IngenHousz Institute, Wageningen, facilitating innovations and large-scale data sharing to enhance photosynthesis research. Jaume Flexas announced *Plant Ecophysiology*, a new open-access journal of which he is Editor-in-Chief. Despite being an important discipline in which photosynthesis measurements have traditionally been central, plant ecophysiology has never before had a dedicated journal of its own. Its unique feature is compensating reviewers—a long-standing point of discussion among the scientific community.

In this review, we synthesize a selection of advancements presented at the congress that, among other topics, are crucial for food and energy security and sustainability in the coming years, as viewed from the authors' perspective. The selection includes optimizing light harvesting and carbon fixation, improving photosynthesis modeling across the scales through mesophyll conductance, and harnessing the natural variability of photosynthesis.

2 TRANSFORMING LIGHT HARVESTING: FROM LEAF TO CANOPY LEVEL OPTIMIZATION

Evolution has not optimized the key components of light harvesting for maximum carbon gain in dense crop monocultures where competitor species are controlled by various crop management practices. Light use efficiency in such systems remains notably low, with only about 1% of light energy being converted into biomass (Blankenship et al., 2011). Addressing this inefficiency requires shifting from enhancing performance at the leaf level of individual plants to rethinking light harvesting across the entire canopy. Several talks focused on key areas for improving photosynthesis through enhanced canopy light use efficiency, including increasing the speed of induction or relaxation of rapidly reversible qE component of non-photochemical chlorophyll fluorescence quenching (NPQ), tuning the antenna size and utilizing far-red light. Altogether, these strategies aim to improve photosynthetic carbon assimilation per unit land area, increase nitrogen-use efficiency, mitigate the effects of heat waves from climate change, and enhance plant water use efficiency by altering leaf temperature through modifications in pigment content and light absorption.

QE (sometimes divided into qE and qZ) is a key photoprotective mechanism, but the modeling by Zhou et al. (2004) showed that slow transitions between relaxation and activation states cause a 30% yield loss. Under real field conditions increasing their speed increased biomass by 15% (De Souza et al., 2022; Kromdijk et al., 2016) and in dense *Nannochloropsis* cultures (Perin et al., 2023) but not in *Arabidopsis* and potato (Garcia-Molina and Leister, 2020; Lehretz et al., 2022), highlighting the complexity of optimizing NPQ across species and the need for alternative approaches.

At this congress work was reported that explored qE at different scales. Johannes Kromdijk demonstrated new genetic targets for breeding crops with improved light-use efficiency under naturally fluctuating field conditions by leveraging natural genetic variation high-definition phenotyping and quantitative trait locus (QTL) mapping in a diverse maize population (MAGIC, Dell'Acqua et al. 2015). This work revealed significant variation in NPQ and photosynthetic efficiency across field seasons, identifying allelic variation in the CP24 protein (LHCB6) on chromosome 10 as a key factor influencing photosynthetic light-use efficiency. The discovery of high natural variability in CP24 is exciting because it highlights the untapped potential of natural genetic variability, motivating further exploration of the genetic underpinnings of those key limiting traits of photosynthesis. Specifically, marker-assisted selection approaches are likely faster to implement, than more profound genetic modifications, so they could accelerate progress in some cases. Lucia Arce-Cubas looked at the effect of different photosynthetic pathways (C3 and C4) on the regulation of NPQ by comparing induction and relaxation across three phylogenetically controlled comparisons. Surprisingly, she found that NPQ relaxation was always faster in the C4 species, which may be explained by the additional sink for ATP and NADPH provided by the C4 cycle.

Traditionally, qE has been treated as a uniform system regarding the speed of induction of the process, quantum efficiency of dissipation, and the functionality its components. Herbert van Amerongen reported their recent results, using spinach as a model, showing that approximately half of qE can switch on/off instantaneously upon the opening and closing of PSII reaction centers. These results highlight the variable speeds of induction of different qE mechanisms (van Amerongen and Chmeliov, 2020). This variability must be accounted when modeling potential yield based on qE responses to ensure accurate outcome emphasizing the need to target the more slowly responding components in modifications and explore their relative contributions to the process.

Peter Jahns demonstrated that the regulation of zeaxanthin epoxidase (ZEP) activity, but not violaxanthin de-epoxidase is central to the regulation of photoprotection and light use

efficiency in multispecies context. ZEP activity is primarily by the amount of ZEP protein (Küster et al., 2023) and by high-light induced down-regulation and degradation of ZEP (Bethmann et al., 2023). Their studies in the multi-species context underscore the flexibility of the ZEP regulatory network, shaped by each species' adaptation to excess light at the timescale of evolution. Sara Natale's comprehensive study on bryophytes showed the plasticity of NPQ was already present in early land plants. Moss species exhibited distinct NPQ activation and relaxation capacities, reflecting their adaptive histories. Under saturating light conditions, some species showed the ability to dynamically modulate and remodel their NPQ induction profiles, highlighting their capacity for photosynthetic acclimation.

Optically dense canopies limit the availability of photosynthetically active photon flux density (PPFD) in lower canopy leaves resulting in an overall reduced number of light quanta but a higher abundance of far-red light. Most of plant photosynthesis relies on visible light (400-700 nm), but far-red light (FRL; >700 nm) has enough energy to induce charge separation in plant photosystems (Schlodder et al., 2014). However, FRL is inefficiently absorbed by plant light-harvesting pigment-binding complexes, and therefore by leaves. Thus, expanding the absorption capabilities in the far-red region could increase the overall canopy light absorption by approximately 20% (Croce et al., 2024). Roberto Bassi shared how his team used cryo-electron microscopy and spectroscopy to study the molecular composition and function of light-harvesting complexes (LHCs) in vascular plants. Their work revealed how these complexes adjust—reflected in various chlorophyll isoforms—to better capture long-wavelength quanta under dynamic light conditions. Specifically, plants with red shifted absorption forms grow better than mutants lacking them in far-red-light enriched conditions. This research provides high-resolution, real-time insights into the structure-function relationships of LHCs, paving the way for synthetic biology approaches to enhance light harvesting. Indeed, the structural data obtained from PSI-LHCI have been used for engineering different LHC proteins reproducing the formation of red-shifted absorption forms in Photosystem II of *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*, that will be analyzed for growth phenotypes in different light intensity and spectral conditions.

Other strategies to increase far-red light absorption rely on the synthesis of the long-wavelength absorbing pigments chlorophyll (chl) *d* and *f* and their incorporation into the photosystems. Although these pigments have so far only been found in cyanobacteria, they provide interesting opportunities and models for tuning photosynthesis in plants and algae. In general, two types of far-red photosynthesis have been described: the *Acaryochloris*-type which replaces nearly all chlorophylls by chl *d* but is limited to the one genus (Miller et al.,

2022) and the more widely distributed type that uses the far-red-light Photoacclimation (FaRLiP) and which uses predominantly chl *a*, developing ~ 10% chl *f* and <1 % chl *d* (Gan et al., 2014). Dennis Nürnberg presented his recent results on the evolution of the genetic regulatory network needed for FaRLiP. A high number of phylogenetically diverse species were used in the study, suggesting that FaRLiP most likely emerged early in cyanobacterial evolution, and has since adapted to diverse low-light and far-red enriched ecological niches. Their findings also indicate that the minimal gene set for FaRLiP is smaller than previously thought (Antonaru et al., 2024). This was later confirmed spectroscopically by Eduard Elias. He demonstrated that certain cyanobacteria perform far-red photosynthesis with chl *f* while operating with a reduced set of FaRLiP components. This simplification could ease adaptation of the FaRLiP system for use in crop-plant engineering. The reduced cluster also highlights FaRLiP's evolutionary plasticity, raising questions about if such streamlined systems can maintain efficiency across diverse environments, and what trade-offs, if any, are involved.

How cyanobacteria utilize far-red photosystems and overcome the 'low energy headroom' remains unclear despite significant spectroscopic and structural advances (Elias et al., 2024). Giovanni Consoli provided critical insights into the long-debated location of chl *f*, the most red shifted isoform known, in Far-Red Photosystem I (FR-PSI)—whether in the reaction center (Nürnberg et al., 2018) or only the antenna (Kato et al., 2020). G.Consoli determined the structure of FR-PSI from *Chroococcidiopsis thermalis* at a resolution that allows to define the locations of chl *f* sites confirming that chl *f* is a redox-active chlorophyll in the reaction center (Consoli et al., 2024), extending fundamental understanding of Far-Red photosynthesis and its functional limits.

An additional approach to simultaneously optimizing canopy photosynthesis and enhancing drought resilience is tuning antenna size to progressively increase as light intensity decreases within the canopy with the aim of having a lower leaf-light absorption by the upper-most leaves of the canopy, and higher leaf-light absorption lower in the canopy. Numerous genetic modifications aimed at reducing antenna size, including lowering chlorophyll content, have been explored across various species. However, the translation of these findings into field-ready, high-performing crops has been challenging. Andrea Persello from Paolo Pesaresi's team has recently characterized the novel pale-green barley mutant *xan-h.chli-1*, which represents a viable homozygous mutation at the *Xan-h* locus encoding for the Mg-chelatase complex subunit *Hv*CHLI. In particular, the Arg298Lys amino-acid substitution in the ATP-binding cleft causes a slight decrease in *Hv*CHLI protein abundance and a marked reduction in Mg-chelatase activity. Under controlled growth conditions, mutant plants display reduced

accumulation of antenna and photosystem core subunits, together with reduced photosystem II quantum yield relative to wild-type under

moderate illumination, and consistently higher than wild-type levels at high light intensities. Moreover, the reduced leaf chlorophyll content is associated with a stable reduction in daily transpiration rate, and slight decreases in total biomass accumulation and water-use efficiency under drought conditions, reminiscent of phenotypic features of wild barley accessions and landraces that thrive under arid climatic conditions (Persello et al., 2024). These findings support the notion that pale green (chlorotic) leaves help plants to thrive under harsh conditions, such as aridity, due to reduced leaf temperature and increased photosynthetic water use efficiency (WUEi). Additionally, it contributes to optimizing the antenna size of the chloroplasts, thereby increasing PPFD (photosynthetic photon flux density) transmittance to the lower canopy leaves, resulting in enhanced whole canopy light interception. However, it should be noted that reduction of chlorophyll content is the result of multiple types of mutations, including those affecting chlorophyll and xanthophyll biosynthesis pathways, LHC protein accumulation, and biogenetic mechanisms. Such impairments might well cause pleiotropic effects and cause photoinhibition or increased sensitivity. Addressing these challenges, Roberto Bassi's lab has successfully performed genome editing of individual chlorophyll binding LHC proteins, generating lines with varying chlorophyll content specifically due to differential accumulation of Lhcb1. This precise approach enables to identify the optimal antenna size for best growth rate while minimizing associated trade-offs. Furthermore, the extra nitrogen that is not invested in pigment-binding proteins can be used for formation of Rubisco and other rate-limiting components of the photosynthetic electron transport chain, thereby increasing the photosynthetic capacity of the lower canopy leaves that in mutant canopies absorb more light (Niinemets, 2023).

3 IMPROVING CARBON FIXATION

Achieving meaningful gains in photosynthesis requires a synergistic improvement of both light harvesting, electron transport and NADPH and ATP synthesis and carbon fixation; in improving carbon fixation, particularly in mitigating Rubisco's inefficiency in discriminating between O_2 and CO_2, as well as enhancing the Calvin-Benson-Bassham (CBB) cycle—both of which hold strong potential for dramatically improving photosynthesis and yields (Raines et al., 2022).

Christine Raines' plenary showed inspiring results of how a simultaneous increase in electron transport rate and the rate of ribulose-15,1-bisphosphate (RuBP) regeneration significantly boosted photosynthesis rates, biomass accumulation, and WUEi in both field and greenhouse conditions. Their approach is distinctive in that simultaneously both the conversion of energy and redox equivalents, and their consumption (RuBP regeneration), are modified. This is a rare dual modification in current strategies that typically target only one of these two key photosynthetic sub-process with the results that the overall improvement might remain moderate. For the efficient functioning of photosynthesis, the energy demands of downstream processes, such as CO_2 fixation in the CBB cycle, must be balanced with the supply provided by the photosynthetic electron transport chain, as highlighted in Raines' plenary. This energy balance is maintained by carefully coordinated feedforward and feedback mechanisms regulatory processes (Matuszyńska et al., 2019). Oliver Ebenhöh, emphasized dynamic theoretical models of photosynthesis, which describe these regulatory feedback mechanisms. Specifically, models show how cyclic electron flow must be tuned to reduce reactive oxygen species production without compromising carbon fixation (Saadat et al., 2021). Dynamic models are invaluable tools for optimizing photosynthesis, particularly in applications that drive advancements in crop improvement. They offer crucial insights into managing trade-offs between key performance parameters—such as yield and stress tolerance— and even overcome them, helping simultaneously enhance plant performance and resilience, particularly in the face of biotic and abiotic stresses exacerbated by climate change.

The concentration of O_2 and CO_2 at the site of carboxylation in C3 plants combined with Rubisco's affinities for CO_2 and O_2 leads to energetically costly photorespiration occurring in C3 plants (and crops) under current atmospheric CO_2 and O_2 partial pressures. Overcoming photorespiration could increase photosynthetic efficiency and concurrently a yield by 20-50% (Walker et al., 2016). Efforts to engineer Rubisco with higher CO_2 specificity have to date met with little success, prompting the exploration of alternative ways of reducing the inefficiency arising from photorespiration (Ort et al., 2015). These include rerouting photorespiratory metabolism via synthetic bypasses, which transform photorespiration into a carbon-positive process (Smith et al., 2023), and employing carbon-concentrating mechanisms (CCM) and pumps creating supra-atmospheric CO_2 concentrations around Rubisco. A key advantage of both approaches is that they require less anatomical intervention compared to implementing the full C4 pathway. After over a decade of active research, several synthetic options for bypassing C3 photorespiration have been developed, with varying levels of promise (Smith et al., 2023). Andreas Weber discussed recent advancements in this area, highlighting the beta-

hydroxy-aspartate cycle as one of the most promising options. Eleonora Traverso from Padova University demonstrated its successful implementation in *Physcomitrium patens*, where it significantly enhanced photosynthetic performance and growth under laboratory conditions.

Alternatively, Urte Schlüter investigated carbon-concentrating mechanisms involving the glycine shuttle, naturally occurring in so-called 'C3-C4 intermediate species' within the *Brassicaceae* family. One clear advantage of implementing intermediate pathways is their ability to function effectively across a wider range of environments than C4, making them valuable for developing climate-resilient crops (Smith et al., 2023). U.Schütler demonstrated a large variation in CO_2 compensation points among the intermediate species, indicating significant differences in the efficiency of operation of the photorespiratory glycine shuttle. However, no evidence of C4-like shuttles was found in the investigated *Brassicaceae* species. The C3-C4 intermediacy was linked to cell-specific glycine decarboxylase complex activity and bundle sheath cells anatomical adaptations, inherently distinguishing them from C3 or C4 species (Schlüter et al., 2023). These findings highlight the complexity of metabolic and anatomical changes required for a fully functional glycine shuttle.

Stefan Timm's recent results on mutants with varying guard cell glycine decarboxylase (GDC) expression suggest that photorespiration occurs not only in mesophyll cells but also in guard cells. Guard cell GDC-modulated lines showed a major impact on stomatal movements, indicating that photorespiration in these specialized cells is critical for regulating stomatal metabolism and behavior. A key question is how optimizing guard cell photorespiration enhances carbon gain under stress and benefits from synthetic pathway integration (Sun et al., 2025).

One promising area for improving photosynthesis is enhancing CO_2 acquisition through the introduction of single-cell CCMs, such as those in cyanobacteria. Kamennaya et al. (2015) demonstrated the successful implementation of a high-flux bicarbonate transporter in cyanobacteria, significantly increasing growth rates and biomass accumulation. This achievement has sparked efforts to transfer similar capabilities into plant chloroplasts (Uehara et al., 2020). Ginga Shimakawa's presentation highlighted a recent collaboration between Japanese, Swiss, and French research institutions, leading to a breakthrough in understanding the atomic structure of the Pyrenoid Shell (PyShell) protein, also revealing its critical role in efficient CO2 fixation (Shimakawa et al., 2024) and offers a blueprint for engineering crop CCMs.

4 MESOPHYLL CONDUCTANCE: ECOPHYSIOLOGICAL IMPLICATIONS, VARABILITY AND MODELING

Improvements to the biochemical machinery of photosynthesis will have limited impact on the realized photosynthesis rate if CO_2 diffusional limitations constrain photosynthesis. Mesophyll conductance (g_m) is a crucial photosynthetic trait quantifying the degree of structural control on photosynthesis. In addition, g_m is a dynamic trait that responds to short-term and long-term changes in environmental factors such as CO_2 concentration, light intensity and temperature due to reasons not fully understood (Márquez and Busch, 2024). Limited g_m causes significant CO_2 drawdown between the intercellular airspace and chloroplasts, contributing up to 70% of total photosynthetic limitation. (Fig. 2; Knauer et al., 2022). Ignoring g_m in photosynthesis calculations can introduce a bias > 30 % in the estimates of the maximum velocity of carboxylation causing erroneous model predictions at leaf to biome levels (Knauer et al., 2020). From the perspective of plant response to global change, interspecific differences in g_m have important implications for predicting vegetation distribution in a future CO_2-rich world (Niinemets et al., 2011). Evolutionarily old species and, in general, plants with robust leaf structure and inherently high g_m limitation may benefit from increasing atmospheric CO_2 via rapidly increasing WUEi.

Given the critical role that g_m plays in limiting photosynthesis, it is essential to incorporate g_m into the Farquhar et al. (1980) model (Farquhar et al., 1980; Márquez and Busch, 2024; Rogers et al., 2017). However, this is challenging because g_m is highly variable and not only difficult to measure but also difficult to predict (Knauer et al., 2020). As demonstrated by Tiina Tosens, even within the same functional group (e.g., gymnosperms) the maximum g_m as determined by inherent variations in leaf anatomy can vary by as much as 30-fold (ranging from 10 to 300 mmol m^{-2} s^{-1}) (Knauer et al., 2022). A current topic of debate is whether g_m could be linked to stomatal conductance (g_s). T.Tosens on a large number of phylogenetically divergent species, and by F.Busch over short-term environmental stress episodes, demonstrated that g_m and g_s do not necessarily correlate, making g_s an unreliable proxy for g_m (Márquez and Busch, 2024). Jaume Flexas´ plenary talk highlighted that the evolution of land plants has been toward balancing limitations related to stomata, mesophyll, and biochemistry (Gago et al., 2019). However, the lack of a functional relationship between g_s and g_m implies the two diffusion pathways determining photosynthetic water use efficiency vary independently, and accordingly different engineering approaches are needed to enhance photosynthesis by modifications of g_s and g_m.

Aside from empirical approaches (e.g., Knauer et al. 2020), there are two primary ways to integrate g_m into the Farquhar et al. (1980) model mechanistically, linking it to leaf anatomy via anatomical model (Tomás et al. 2013) or basing it on leaf biochemistry and physiology (Tholen et al. 2012)- two approaches that may be mutually compatible. F.Busch demonstrated recent progress in combining the two approaches to reliably simulate gas exchange under short-term environmental changes. His approach accounts for the subcellular CO_2 diffusion pathways across different compartments and considers diffusion of both CO_2 and bicarbonate. The resulting g_m model can be fully integrated into the Farquhar et al. model and is thus far the only approach that can successfully simulate instantaneous g_m responses to light, temperature, and CO_2. Accurate predictions of g_m under fluctuating environments will undoubtedly improve photosynthesis estimates in any process-based model. T.Tosens showed that the anatomical model had a high predictive power in predicting the maximum g_m in evolutionarily old species with robust leaf structure and particularly thick cell walls; angiosperms with thinner cell walls exhibited greater variability. This variability likely reflects the variation in cell wall porosities at given cell wall thickness and increasing importance of other factor such as CO_2/bicarbonate equilibrium and carbon flux rate itself. T.Tosens and J.Flexas both demonstrated that two anatomical traits—mesophyll cell wall thickness (T_{cw}) and chloroplast surface area exposed to intercellular airspace per leaf area (S_c)—consistently drive g_m variability across the land plant phylogeny and environments (Fig. 2B,E). Consistent with this idea, Salesse-Smith et al. (2024) recently achieved improved photosynthesis in field-grown tobacco by means of over-expressing an *Arabidopsis* gene related with pectin methyl-esterification, which results in reduced T_{cw} and increased cell wall porosity. Thus, understanding the structure of cell walls and ways of changing it offers interesting ways of improving photosynthesis.

5 LEARNING FROM NATURE: HARNESSING NATURAL VARIABILITY FOR PHOTOSYNTHESIS IMPROVEMENTS

Harnessing natural variability offers a promising approach to improving both crop resilience and photosynthetic efficiency, especially as climate change imposes increasingly harsh conditions on agricultural systems. The genetic diversity found in (wild) plant species is a vast reservoir of traits that can be leveraged via QTL mapping but the challenge lies in effectively identifying, understanding, and incorporating these traits into modern breeding programs (Theeuwen et al., 2022), especially considering the trade-offs between photosynthetic capacity

and stress tolerance in nature (Onoda et al., 2017). David Kramer's keynote talk highlighted the power of using big data for easily assessed photosynthetic traits that can be measured in the field to gain an insight into the importance of different environmental fluctuations on canopy photosynthetic variation across the globe. Lab characterisation of photosynthesis typically only invesigates the influence of limited range of combinations of environmental drivers on photosynthesis and so will fail to evaluate the impact on photosynthesis of the full range of combinations environmental drivers occuring in the field. Identification of the global environmental variation in photosynthesis across time and scale, and the mechanisms responsible for this variation, allows development of genetic engineering and other breeding approaches (genomic selection, conventional marker assisted breeding, gene editing) specifically targeting those processes that are most influential in determining crop photosynthesis. To this can be added the need to develop the tools to reliable assessment of regional and global photosynthesis using remote sensing methods such as sun-induced fluorescence. J.Flexas' keynote presentation demonstrated how plants from different environments overcome these trade-offs in nature (Fig 1A,B). This was based on combined sampling in extreme environments and across axes of phylogenetic variation. It highlights how such "learning from nature" approach offers strategies for improving crop productivity while simultaneously maintaining stress tolerance. He further noted the limitations of commercially designed portable gas-exchange devices when measuring quirky foliage in extreme environments (e.g., temperature, atmospheric pressure). Nonetheless, with creative approaches, photosynthesis can be measured in almost any plant. J.Flexas showed this through a broad range of plants, from bryophytes to angiosperms, studied in remote locations like Antarctica and the high elevations of the Andes. He further demonstrated that the ratio between electron transport rate (ETR) and either net or gross CO_2 assimilation (i.e., the ETR/A ratio) can be used to assess photosynthesis depression without the prior knowledge of maximum photosynthetic rate of given species (Perera-Castro and Flexas 2023). This ratio also serves as a quick test that your instrument is functioning properly, which is particularly useful when working at remote sites away from more precise calibration tests. As an example, using this ratio Flexas et al. (2022) were able to show that in dry steppes in Western China, C4 species were counterintuitively far more photosynthetically depressed than co-existing C3 species after the dry season. Ian Coghill addressed some of these methodological challenges by presenting a custom-built fluorescence imaging system designed for tiny foliage and nonstandard conditions.

To understand the resilience of photosynthetic systems under extreme aridity, Nina Kamennaya compared the photosynthetic growth rates of cyanobacteria and algae coexisting in soil crusts of extreme drylands. Remarkably, they found that even in hyper-arid sites, over 95% of chlorophyll-containing microorganisms remained photosynthetically active. Regardless of the aridity level, cyanobacteria exhibited higher biovolume-specific CO_2 fixation rates than algae. This finding could explain the dominance of cyanobacteria over algae in crusts (Büdel et al., 2016). The team is now working to elucidate the specific mechanisms underpinning the higher efficiency of cyanobacterial photosynthesis compared to algal photosynthesis.

As demonstrated by several conference presentations, natural genetic variation offers a vast untapped potential to improve photosynthesis, either by selecting traits or expanding our understanding of the trade-offs underlying this process. Tom Theeuwen presented a new roadmap for using genetics to improve photosynthesis based on natural variation. Using *A. thaliana* as a model system, he showed a novel method to systematically explore the effect of both nuclear and organellar genetic variation on photosynthetic processes (Theeuwen et al., 2024; Theeuwen et al., 2022). The populations were grown in highly dynamic environmental conditions in climate chambers and in gauze tunnels, revealing that the identification of QTLs in the nuclear, chloroplast and mitochondrial genomes is highly dependent on both the environmental conditions and time. Moreover, the genes underlying these QTLs encode for parts of mechanisms that are outside the core photosynthetic processes. These findings emphasize that there is ample genetic variation for photosynthesis, but that we need to develop novel sensors and methods to phenotype photosynthetic processes in field grown plants throughout time and over a range of environmental conditions if we are, to identify genetic variation with the potential to improve photosynthesis and yield.

6 FUTURE PERSPECTIVES

The Congress highlighted rapid advancements in photosynthesis research, driven by technological innovations and the urgent need for climate change solutions. In past decades, the cutting edge of photosynthesis focussed on the mechanisms, but now there´s a shift toward understanding more of the variation, its genetic basis, and the usefulness or adaptiveness of this variation to the diverse photosynthetic organisms found in the biosphere. The Congress themes reflect this shift, focusing more on the "why" of photosynthesis than the "how." Significant

progress has been made in understanding photosynthesis' key components, paving the way for "smart canopies" optimizing processes at the leaf level, tailored to specific microenvironments. Phenotype verification is a critical next step to ensure these advancements translate into real-world agricultural solutions. Developing advanced phenotyping facilities, particularly if supported by governmental or European institutions, would play a pivotal role in addressing this need. Establishing standardized protocols and conditions for assessing photosynthetic efficiency, biomass production and resilience would be the first step toward a unified understanding trait performance across diverse environments.

Mutants, gene editing, and genetic engineering have shown their potential to enhance photosynthesis efficiency, achieving biomass increases ranging from 10% to 68%, primarily in controlled settings and small-scale field trials. However, there is currently no data on the combined adoption of these approaches, despite the expectation that modifying crucial pathways in plant metabolism—such as light conversion and carbon fixation—will involve complex interdependent effects requiring further investigation. Now is the time to integrate these strategies into elite crop germplasms and rigorously evaluate their performance in real-world cultivation conditions, focusing on total biomass accumulation and yield. It remains necessary move towards measuring photosynthesis in situ and over the longer term if we are to understand the ways that photosynthesis does or does not function well in nature. This will require new instruments and approaches to viewing and evaluating photosynthesis so we can describe and identify the axes of photosynthetic adaptiveness.

Harnessing the vast genetic diversity of natural traits through modern genetic tools offers strong potential coming years. To fully realize this potential, tecnological advancements are required for phenotype screening in real-world field conditions. The open science platform will play a pivotal role in fostering innovation, enabling interdisciplinary collaborations, facilitating large-scale data sharing, and driving transformative advancements in crop improvement across diverse environments. Refining and applying dynamic modeling will be important part of improvements to ensure that sink and source capacities function holistically within the plant system under diverse stress conditions.

Identifying plants and mechanisms how plants overcome the trade-off between resilience and photosynthesis in extreme environments likely provides valuable insights for crop improvements. Advancing from model plants and standard conditions requires manufacturers to innovate advanced gas-exchange equipment. Also, exploring non-foliar photosynthetic mechanisms (stem, bark, wood) may provide interesting avenues (Natale et al.,2024).

Reliable integration of mesophyll conductance into photosynthesis simulations is expected in the near future likely, leading to improved photosynthesis estimations across the scales. As research accelerates, the upcoming Liverpool congress promises exciting developments in these and many other areas of photosynthesis.

AUTHOR CONTRIBUTIONS

T. Tosens outlined the first draft of manuscript. All authors contributed in writing and editing of the manuscript and approved the final version.

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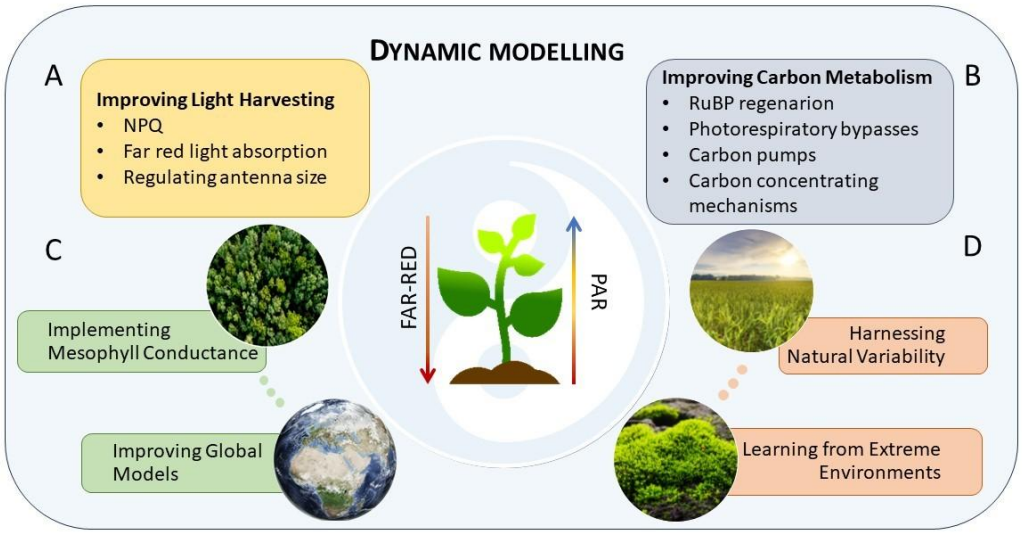


Figure 1. Conceptual framework of photosynthetic mechanisms and improvement strategies discussed in this mini-review. Photosynthetic enhancements can be achieved through synthetic biology, focusing on light harvesting (**A**) and carbon metabolism (**B**), or by harnessing natural variability (**D**)—either by exploring key photosynthetic traits in crop varieties or identifying unique trait combinations in plants adapted to extreme environments that enhance both photosynthesis and resilience. The central illustration features a field crop within a Yin-Yang symbol, emphasizing the holistic integration of these improvement strategies, guided by dynamic modeling. Upper leaves are paler illustrating canopy level improvement strategy where leaves are tailored in their microenvironments as upper leaves have smaller antenna than lower leaves to increase light penetration to canopy lower leaves. Additionally, incorporating mesophyll conductance across multiple modeling scales (C)—from leaf to ecosystem and Earth system models—is crucial for achieving accurate predictions. Abbreviations: NPQ – Non-Photochemical Quenching, RuBP - Ribulose 1,5-bisphosphate, PAR – Photosynthetically Active Radiation.

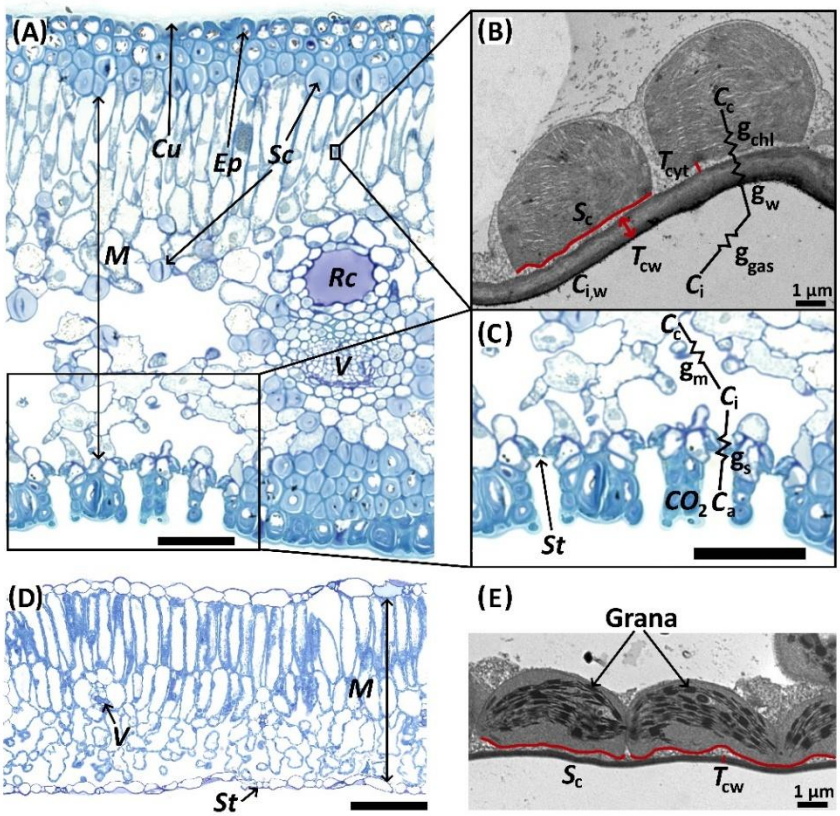


FIGURE 2. Comparison of semi-thin and ultrathin leaf cross-sections of the gymnosperm *Zamia furfuracea* (A-C) and the crop *Helianthus annuus* (D, E), illustrating the CO_2 diffusion pathway from ambient air to chloroplasts in two species with contrasting life strategies. Stomatal conductance (g_s) regulates the CO_2 concentration drop between ambient air (C_a) and substomatal cavities (C_i), while mesophyll conductance (g_m) controls the CO_2 drawdown between Ci and the site of carboxylation (chloroplast Cc). gm is composed of gas-phase (g_{gas}) and liquid-phase (g_{liq}) conductance. g_{gas}, which depends on mesophyll thickness and airspace porosity, determines the drop between *C*i and the CO_2 concentration on the outer surface of the cell wall (*C*i,w). In turn, g_{liq} controls the CO_2 drawdown from Ci,w to Cc, being influenced by multiple anatomical barriers, including cell wall conductance (g_w), the plasma membrane, cytoplasm, chloroplast envelope, and chloroplast stroma (g_{chl}). These barriers, defined by their thickness and porosity, largely determine the maximum gm for a given species. Notable interspecific variability in gm is driven by key anatomical traits such as cell wall thickness *(*Tcw) and chloroplast surface area exposed to the mesophyll airspace (Sc) (B and E). gm can also vary in the short term, as highlighted by F. Busch, due to its composite nature, which integrates multiple partial conductances (e.g., g_w and g_{chl}). *Z. furfuracea* represents the low-return end of the leaf economic spectrum (LES, see Onoda et al., 2018), prioritizing carbon and nitrogen investment in defense tissues (e.g., resin channels, sclereids, and a thick cuticle). This strategy results in thick lignified mesophyll cell walls, increasing the CO_2 diffusion path length and thereby limiting g_m. In contrast, fast-growing crops at high return end of LES, such as *H. annuus* invest primarily in photosynthetic structures, maximizing photosynthesis rates at the expense of stress tolerance. Can we identify a species that combines high photosynthesis rates with strong resilience? Abbreviations: Cu- cuticle; Ep- epidermis; SC- sclerids; RC- resin channels; V- vein; M-mesophyll; St- stomata. Scale bars: A, C and D 100 µm, B and E 1 µm. Unpublished images by T.T.