

Photosynthesis Phenotyping: Where Are We and How Should We Move Forward?

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Abstract: Photosynthesis phenotyping is increasingly important for crop improvement, but its main limitation is not measurement throughput alone. It is the physiological interpretation of scalable signals. Direct methods, including gas exchange, CO₂ response analysis, isotope-based approaches, and canopy flux measurements, define photosynthetic carbon assimilation and capacity, but remain difficult to deploy at scale. Optical and imaging approaches provide high-throughput observations of plant function, but they mainly capture indirect, context-dependent signals. We argue that a core challenge in the field is the frequent conflation of proxy measurements with photosynthetic carbon assimilation. Scalable traits often reflect integrated physiological states rather than carbon flux itself, and their relationship to photosynthesis depends on environmental context, biological scale, and the processes limiting carbon gain. Progress will therefore require treating scalable measurements as inferential signals rather than direct physiological quantities. It will also require stronger physiological benchmarks and validation frameworks across genotypes, environments, developmental stages, and spatial scales.

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1. Introduction

Photosynthesis converts light energy into chemical energy and atmospheric CO₂ into plant biomass. In crops, this process underpins yield formation, resource-use efficiency, and tolerance of environmental fluctuation (Zhu, Long, & Ort, 2010). Improving photosynthesis has therefore re-emerged as a major target for crop improvement, particularly as further gains from selection for harvest index and plant architecture appear increasingly constrained (Araus et al., 2021). Climate change has further intensified this interest (Baslam et al., 2020). Rising temperature, altered precipitation patterns, higher vapour pressure deficit, and more frequent drought and heat events can restrict photosynthetic carbon assimilation through stomatal closure, mesophyll diffusion limitation, photochemical regulation, Rubisco activity, respiration, and sink demand (Scafaro et al., 2023; Violet-Chabrand et al., 2017).

Despite its central role, photosynthesis remains difficult to use as a selection trait in crop breeding (Taylor, 2024). A major obstacle is scale. Leaf-level measurements provide mechanistic information on stomatal, mesophyll, biochemical, and photochemical limitations, but they often represent only one leaf, one developmental stage under given environmental conditions (Araus et al., 2021). They therefore do not necessarily predict seasonal canopy carbon gain or final yield (Robles-Zazueta et al., 2022). Canopy-level measurements are more directly related to crop growth, but they often provide less information about the physiological processes underlying genotypic variation (Long et al., 2022). This mismatch between mechanistic resolution and agronomic relevance has limited the practical use of photosynthetic traits in selection (Song & Zhu, 2024).

The second difficulty is throughput. Direct or semi-direct methods, including leaf gas exchange, CO₂ response



curves, isotope-based approaches, and canopy flux measurements, are physiologically informative but slow, technically demanding, and difficult to apply across large breeding populations, diversity panels, or multi-environment field trials (Araus & Cairns, 2014; Du et al., 2020; Song & Zhu, 2024). High-throughput phenotyping offers a way to overcome part of this limitation. Chlorophyll fluorescence measurements, spectral reflectance, thermal imaging measurements, structural imaging, and solar-induced chlorophyll fluorescence (SIF) measurements can capture spatial variation, temporal dynamics, and genotype-by-environment responses at scales that conventional gas exchange cannot reach (Araus et al., 2022; Fu et al., 2022; Siebers et al., 2021; Song & Zhu, 2024).

However, higher throughput does not by itself solve the problem of photosynthesis phenotyping (Fu et al., 2022; Song & Zhu, 2024). Most scalable measurements are proxies rather than direct estimates of photosynthetic carbon assimilation (Aasen et al., 2019; Fu et al., 2022; Song & Zhu, 2024). They may reflect photochemical efficiency, pigment or nitrogen status, canopy structure, water status, stomatal behaviour, stress responses, or combinations of these traits (Araus et al., 2022; Berger et al., 2010; Feng et al., 2021; Pineda, Barón, & Pérez-Bueno, 2020). Their value therefore depends on whether the measured signal can be linked to a clearly defined photosynthetic phenotype and to the carbon-flux trait of interest (Camino et al., 2019; Song & Zhu, 2024). The central challenge in photosynthesis phenotyping is thus not simply how to measure faster, but how to interpret scalable signals physiologically and how to anchor them to direct measurements.

In this review, we examine photosynthesis phenotyping as a proxy problem. We first clarify what is meant by a photosynthesis phenotype across biological scales. We then summarise direct and semi-direct methods for estimating photosynthesis, including gas exchange, isotope-based approaches, and canopy-scale flux measurements, emphasising their role as physiological reference methods. Next, we assess major scalable optical and imaging approaches, including chlorophyll fluorescence measurements, spectral reflectance measurements, thermal imaging, structural imaging, and SIF measurements. Finally, we discuss how scalable sensing can be made more physiologically interpretable through direct anchoring, dynamic measurement protocols, multi-sensor integration, and mechanistic modelling.

2. What Do We Mean by A Photosynthesis Phenotype?

A major difficulty in photosynthesis phenotyping is that photosynthesis is not a single trait (Song & Zhu, 2024). Depending on the method, scale, and research question, the measured phenotype may refer to net CO₂

assimilation (A_{net}), photosynthetic capacity, photochemical efficiency, CO₂ diffusion, water-use efficiency (WUE), canopy carbon gain, biomass accumulation, or yield (Araus et al., 2021; Fu et al., 2022; Song & Zhu, 2024; Zhu, Long, & Ort, 2010). These traits are related, but they are not interchangeable. A phenotyping method should therefore be evaluated only after the target phenotype has been clearly defined. Photosynthetic carbon assimilation is also highly context-dependent. Net assimilation can vary within a single leaf, across the day, among developmental stages, and across vertical canopy layers (Figure 1A–D). Moreover, leaf-level carbon exchange does not necessarily scale directly to whole-plant carbon exchange (Figure 1E). These sources of variation illustrate why photosynthesis should not be treated as a single phenotype and why the target trait must be defined before selecting a phenotyping method.

For practical phenotyping, photosynthesis-related traits can be grouped into four categories. The first includes carbon-flux traits, such as net leaf-level CO₂ assimilation commonly denoted as A_{net} (Siebers et al., 2021), whole-plant CO₂ exchange, canopy photosynthesis, and gross primary productivity (GPP). These traits are closest to the process of interest because they describe realised carbon uptake under defined environmental conditions. They provide the most direct reference for evaluating photosynthetic performance, although their values depend strongly on light, CO₂ concentration, temperature, vapour pressure deficit within the gas exchange chambers, developmental stage, and recent environmental history (Violet-Chabrand et al., 2017).

The second category includes capacity traits, such as maximum Rubisco carboxylation capacity (V_{cmax}), maximum electron-transport capacity (J_{max}), and triose phosphate utilisation (TPU) limitation (De Souza & Long, 2018; Maroco et al., 2002; Miao et al., 2009). These traits describe the biochemical potential for photosynthesis rather than the instantaneous rate achieved under a particular field condition (Miao et al., 2009; Vaz et al., 2011). They are useful for comparing genotypes under standardised or model-defined conditions, but they are derived from response curves and depend on assumptions about mesophyll conductance, respiration, temperature responses, and fitting procedures (Duursma, 2015; Hogewoning et al., 2010; Xiong et al., 2017).

The third category includes component-process traits that regulate or constrain carbon assimilation (Kalaji et al., 2016; Li et al., 2021; Xiong et al., 2017; Xiong et al., 2015b). These include stomatal conductance (g_s), mesophyll conductance (g_m), photochemical efficiency, electron transport, non-photochemical quenching, pigment status, leaf nitrogen, water status, and canopy light interception (Kaiser et al., 2018; Kalaji et al., 2016; Momayyezi et al., 2022; Xiong et al., 2017; Ye et al., 2026). Such traits help explain why photosynthesis differs among genotypes, treatments, or environments (Flood,

Harbinson, & Aarts, 2011; Sales et al., 2022). However, they should not be interpreted as direct measures of

carbon assimilation unless their relationship to carbon flux has been established (Siebers et al., 2021).

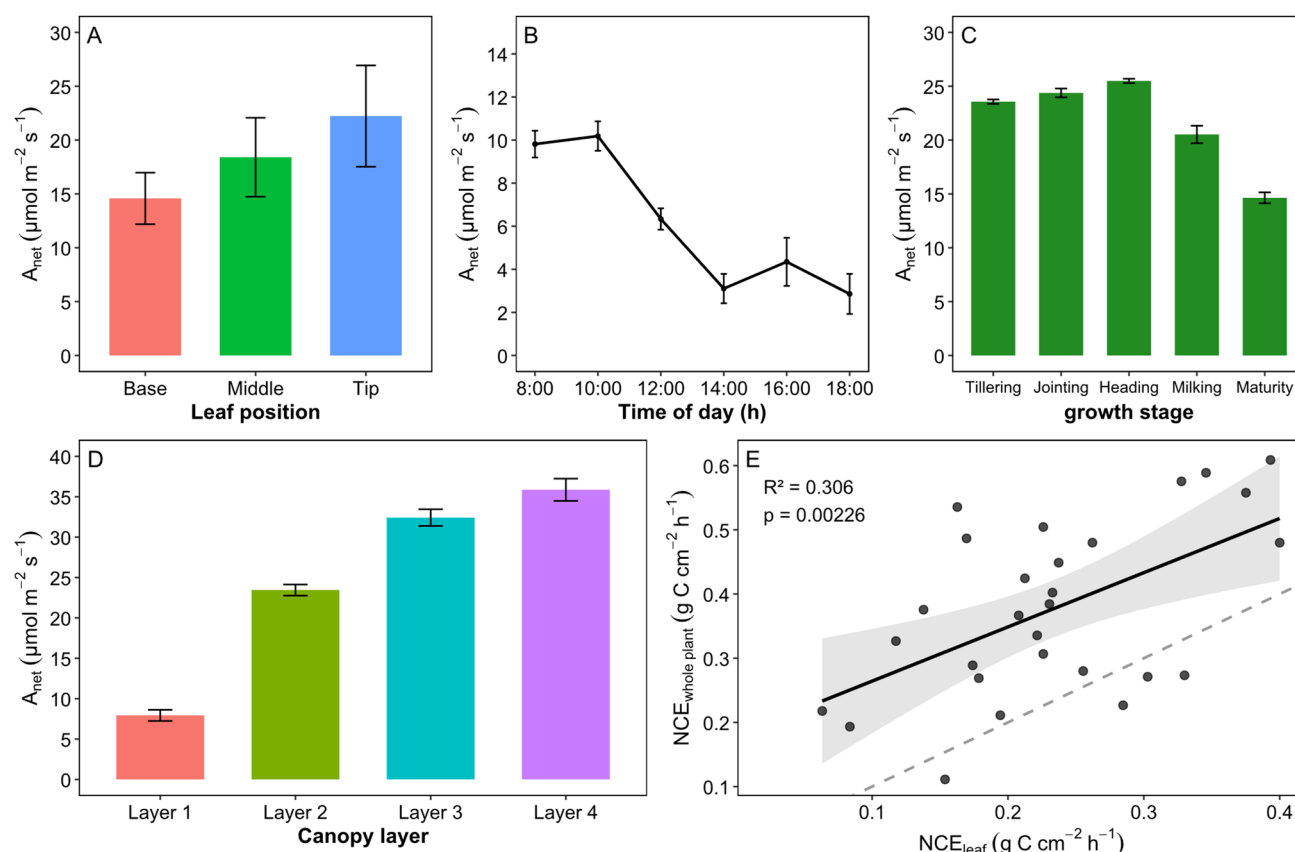


Figure 1. Context dependence of photosynthetic carbon assimilation across tissues, time, development, canopy position, and biological scale. **(A)** Net CO₂ assimilation (A_{net}) measured at the base, middle, and tip of maize leaves. **(B)** Diurnal variation in *Camellia oleifera* net photosynthetic rate during the daytime. **(C)** Variation in leaf net photosynthetic rate across five rice growth stages, including tillering, jointing, heading, milking, and maturity. **(D)** Vertical variation in carbon assimilation across four canopy layers (Layer 1 to Layer 4 from bottom to top). **(E)** Relationship between leaf-level and whole-plant net carbon exchange (NCE). For panels **A**, **C**, and **D**, bars represent mean $\pm$ standard error. For panel **B**, points and error bars represent mean $\pm$ standard error where available. For panel **E**, the line indicates the fitted linear regression, the shaded band indicates the 95% confidence interval, and the grey dashed line indicates the 1:1 reference line representing perfect agreement between leaf-level and whole-plant NCE. Data were digitised from published studies using GetData Graph Digitizer 2.22. All panels were visualised using R version 4.3.2. Panel **A** was digitised from Liu et al. (2025), panel **B** from Zhang et al. (2023), panel **C** from Wang et al. (2020), panel **D** from Yan et al. (2024), and panel **E** from Escalona et al. (2016).

The fourth category includes integrated performance traits, such as radiation-use efficiency (RUE), seasonal canopy carbon gain, biomass, and yield (Deng et al., 2026; Slattery & Ort, 2021; Song & Zhu, 2024; Zhu, Long, & Ort, 2010). These traits are often most relevant to crop improvement, but they are downstream outcomes of photosynthesis rather than photosynthesis itself (Xiong, 2024). They integrate leaf area development, canopy architecture, phenology, respiration, carbon allocation, nutrient uptake, source-sink balance, stress escape, and harvest index (Deng et al., 2026; Rahman et al., 2018; Reynolds et al., 2012; Slattery & Ort, 2021). A genotype with higher leaf photosynthetic capacity will not necessarily produce higher yield if additional assimilates are not captured, retained, transported, or used by sink tissues (Araus et al., 2021).

This distinction among carbon-flux traits, capacity traits, component-process traits, and integrated performance traits is important for interpreting high-throughput measurements (Fu et al., 2022; Song & Zhu, 2024). A scalable signal may be useful even when it does not directly estimate CO₂ assimilation, provided that its physiological meaning is clear (Aasen et al., 2019; Siebers et al., 2021). Conversely, a proxy can be misleading when it is treated as a general photosynthesis phenotype without specifying whether it represents carbon uptake, photosynthetic capacity, photochemical regulation, water status, canopy structure, or downstream growth (Wohlfahrt et al., 2026). Thus, photosynthesis phenotyping should begin not with the sensor, but with the physiological question: which photosynthetic trait is being targeted, at which scale, and for what biological or breeding purpose?

3. Direct and Semi-Direct Methods for Estimating Photosynthesis

Direct and semi-direct physiological methods provide the reference frame for photosynthesis phenotyping (Fu et al., 2022; Siebers et al., 2021; Song & Zhu, 2024). They are rarely practical as stand-alone tools for screening large breeding populations, diversity panels, or multi-environment field trials, but they define the carbon-flux traits against which scalable proxies should be evaluated (Du et al., 2020; Song & Zhu, 2024; Taylor, 2024). Their value therefore lies not only in measuring photosynthesis, but also in clarifying what optical, thermal, spectral, or imaging signals actually represent.

3.1. Gas-exchange-based approaches

Gas exchange remains the principal direct approach for quantifying photosynthetic carbon assimilation (Busch et al., 2024). In a typical measurement, a portable photosynthesis system encloses part of a leaf in a chamber and estimates CO₂ assimilation and transpiration from the differences in CO₂ and water vapour concentrations between incoming and outgoing air streams (Hogewoning et al., 2010; Siebers et al., 2021; Song & Zhu, 2024). A single gas-exchange measurement provides instantaneous net assimilation, but this measurement alone does not reveal which biochemical or diffusional processes are limiting photosynthesis (Song & Zhu, 2024). Gas-exchange-based response curves provide additional physiological resolution. In CO₂ response analysis, assimilation is measured across a sequence of intercellular CO₂ concentrations (C_i), generating an $A-C_i$ curve. When fitted with biochemical models of C₃ photosynthesis (Farquhar, von Caemmerer, & Berry, 1980), these curves can be used to estimate photosynthetic capacity parameters, including V_{cmax} , J_{max} , and TPU limitation (De Souza & Long, 2018; Miao et al., 2009; Vaz et al., 2011). Thus, gas exchange can provide both instantaneous rate traits, such as A_{net} , and model-derived capacity traits, such as V_{cmax} and J_{max} . The former describe realised photosynthetic performance under the measurement environment, whereas the latter describe potential biochemical capacity under standardised or model-defined conditions.

Gas exchange also provides access to diffusional limitations. Stomatal conductance can be estimated routinely from CO₂ and water vapour exchange and is central to photosynthesis phenotyping because it links carbon gain with water loss (Song & Zhu, 2024). Under favourable conditions, higher stomatal conductance may support higher assimilation, whereas under drought or high vapour pressure deficit it may increase water loss and reduce stress tolerance (Casari et al., 2019; Sales et al., 2022). Mesophyll conductance, which describes CO₂ diffusion from the substomatal cavity to the chloroplast, is more difficult to estimate, but it matters because it determines the CO₂ concentration at Rubisco (Xiong et al.,

2017; Xiong et al., 2015a). It can be inferred by combining gas exchange with chlorophyll fluorescence, carbon isotope discrimination, or modelling approaches (Pathare et al., 2020; Xiong et al., 2015a; Xiong et al., 2017). Because mesophyll conductance remains difficult to phenotype at scale (Berghuijs et al., 2016; Pathare et al., 2020), it is a hidden source of photosynthetic variation that optical methods may not capture.

Gas-exchange approaches can also be scaled beyond a single leaf (Siebers et al., 2021; Song & Zhu, 2024). Whole-plant and canopy gas-exchange systems enclose shoots, plants, or crop stands and measure integrated CO₂ exchange. These systems are valuable because crop productivity depends on canopy carbon gain rather than photosynthesis in a single leaf (Araus et al., 2021; Zhu, Long, & Ort, 2010). They capture the effects of leaf area, canopy architecture, developmental stage, leaf age distribution, and the combined contribution of multiple leaves (Iio et al., 2009; Song & Zhu, 2024). A genotype with moderate leaf photosynthetic capacity but better canopy structure, slower senescence, or improved light distribution may achieve higher seasonal carbon gain than a genotype with high photosynthesis in one upper leaf (Molero & Reynolds, 2020; Reynolds et al., 2012; Slattery & Ort, 2021). Canopy gas-exchange measurements integrate CO₂ fluxes across leaves and, when recorded continuously or repeatedly, can quantify carbon exchange over extended periods. Routine measurements of individual leaves may capture only a short time window, although dedicated leaf-level protocols can also resolve temporal dynamics. Rapid physiological responses, such as those to fluctuating light, may be obscured in canopy records by spatial integration and the response time of the measurement system.

The main limitation of gas exchange is throughput. Reliable measurements require time, technical skill, careful environmental control, and attention to chamber artifacts (Du et al., 2020; Hogewoning et al., 2010; Song & Zhu, 2024). Leaf measurements can be affected by many factors including chamber leaks, insufficient acclimation, poor sealing, inaccurate leaf area, unstable vapour pressure deficit, stomatal patchiness, and temperature variation (Hogewoning et al., 2010; Xiong et al., 2015a). $A-C_i$ curves are slower and model-dependent (Aminizadeh et al., 2024; Duursma, 2015; Miao et al., 2009; Vaz et al., 2011). Estimates of V_{cmax} and J_{max} can be biased by assumptions about mesophyll conductance, day respiration, TPU limitation, temperature responses, and fitting procedure (Scafaro et al., 2023). Whole-plant and canopy chambers introduce additional artifacts by altering temperature, humidity, wind speed, boundary layer conductance, and light environment. Rapid $A-C_i$ protocols, automated gas-exchange systems, and multiplexed chambers can improve throughput, but gas exchange is still unlikely to become a stand-alone screening method for very large breeding populations (Du

et al., 2020; Song & Zhu, 2024). Its strongest role is to provide physiological anchors and training data for scalable phenotyping models (Faralli et al., 2024; Lazarević et al., 2026; Lu et al., 2025; Sharma et al., 2024).

3.2. Labelling and isotope-based approaches

Labelling and isotope-based approaches provide information that is difficult to obtain from standard gas exchange alone (Atkins & Canvin, 1971; Liu et al., 2025). Rather than measuring only instantaneous CO₂ uptake, these methods can integrate photosynthetic behaviour over time, partition carbon and water-use processes, or trace the fate of recently assimilated carbon (Medlyn et al., 2017). They are therefore useful for validating proxy signals and for identifying the physiological mechanisms behind phenotypic variation.

Carbon isotope discrimination is widely used to infer the balance between CO₂ supply and carboxylation demand (Cornwell et al., 2018). Because discrimination against ¹³C is affected by the ratio of internal to ambient CO₂ concentration, it provides an integrated indicator of intrinsic water-use efficiency (Cernusak, 2020). In natural-abundance studies, the carbon isotope composition measured in plant dry matter can reflect photosynthetic and stomatal behaviour integrated over the period of tissue formation (Cernusak, Pate, & Farquhar, 2002; Wang, Zhang, & Li, 2007). This makes isotope traits useful for linking photosynthesis, stomatal regulation, and adaptation to water-limited environments.

In isotope approaches coupled with gas-exchange measurements, the concentration and carbon isotope composition of CO₂ are measured in air entering and leaving the leaf chamber. These measurements are used to calculate photosynthetic carbon isotope discrimination, which can be interpreted alongside gas-exchange data using appropriate models to estimate mesophyll conductance and investigate the processes underlying CO₂ exchange (Diao et al., 2024; Ubierna & Farquhar, 2014). These measurements can help distinguish the physiological bases of similar net CO₂ assimilation rates. For example, *Brassica nigra* and *B. rapa* exhibited similarly high assimilation rates per unit leaf area when grown and measured under high light. *B. nigra* had higher biochemical capacity, whereas *B. rapa* had higher stomatal and mesophyll conductance. Mesophyll conductance estimates derived from carbon isotope discrimination supported the trends obtained from gas-exchange and chlorophyll fluorescence measurements (Retta et al., 2024).

By measuring ¹³C enrichment in plant tissues sampled after ¹³CO₂ labelling, these methods can trace the movement of recently assimilated carbon from source leaves to other organs and storage pools and reveal differences in carbon allocation among genotypes or environments (Nogués et al., 2006; Xu et al., 2023). This is important because increased leaf photosynthesis will

improve yield only if additional carbon can be exported, transported, and used by sink tissues (Holland et al., 2019; Kilbane et al., 2025). Labelling approaches therefore help connect photosynthetic rate with carbon allocation, sink strength, and biomass formation (Xu et al., 2023). Labelling and isotope methods require specialised instrumentation and careful sampling design and can involve substantial analytical costs. Interpreting isotope-labelling results also requires expertise in carbon metabolism and allocation, particularly when using measured isotopic enrichment to assess how newly assimilated carbon is distributed among metabolites and structural components.

4. Scalable Optical and Imaging Proxies

Current high-throughput phenotyping of photosynthesis relies heavily on optical and imaging methods because they are rapid, non-destructive, and deployable from leaves to canopies (Fu et al., 2022; Li et al., 2014; Song & Zhu, 2024). These methods measure signals associated with photochemistry, pigments, nitrogen, water status, energy dissipation, temperature, and canopy structure rather than CO₂ assimilation itself (Araus et al., 2022; Li et al., 2014; Liu et al., 2020; Pineda, Barón, & Pérez-Bueno, 2020). Their value therefore depends on matching each signal to the appropriate photosynthesis-related phenotype, including photochemical performance, biochemical capacity, stomatal limitation, canopy light capture, or integrated crop performance (Araus et al., 2022; Fu et al., 2022; Song & Zhu, 2024). Here, we summarise five major scalable approaches and evaluate where each is most informative.

4.1. Chlorophyll fluorescence

Chlorophyll fluorescence measurements serve as a widely-applied core optical technique for photosynthesis phenotyping (Aasen et al., 2019; Bhagooli et al., 2021; Pérez-Bueno, Pineda, & Barón, 2019). Most chlorophyll fluorescence-derived phenotyping parameters are measured by pulse amplitude modulation (PAM) chlorophyll fluorometry. PAM chlorophyll fluorometry is an active measurement technique employing instrument-generated modulated measuring light and saturating light pulses to quantify photosystem II fluorescence yields under defined light conditions. Chlorophyll fluorescence is used to estimate the partitioning of absorbed light energy among photochemistry, heat dissipation, and chlorophyll fluorescence emission (Murchie & Ruban, 2020; Rapparini et al., 2015). Because these pathways compete, changes in chlorophyll fluorescence provide information about the operating state of photosystem II and the balance between photochemical energy use and non-photochemical dissipation (Krause & Weis, 1991; Rapparini et al., 2015). Common chlorophyll fluorescence parameters include maximum quantum yield of photosystem II (F_v/F_m),

operating efficiency of photosystem II (Φ_{PSII}), electron transport rate (ETR), and non-photochemical quenching (NPQ) (Bhagooli et al., 2021; Kalaji et al., 2016). F_v/F_m estimates the maximum quantum efficiency of photosystem II after dark adaptation and is commonly used to detect photoinhibition or chronic stress injury (Silva-Cancino et al., 2012; Sukhvir et al., 2000). Φ_{PSII} estimates the operating efficiency of photosystem II under light. ETR provides an apparent estimate of electron transport rate, usually calculated from Φ_{PSII} , incident light, leaf absorptance, and assumptions about light partitioning between photosystems (Perera-Castro & Flexas, 2023). NPQ reflects regulated thermal dissipation of excess excitation energy and is widely used as an indicator of photoprotection (Murchie & Ruban, 2020).

For photosynthesis phenotyping, the main strength of chlorophyll fluorescence is not that it directly estimates carbon assimilation, but that it resolves the photochemical component of photosynthesis with high sensitivity (Fu et al., 2022; Pérez-Bueno, Pineda, & Barón, 2019; Song & Zhu, 2024). This is especially useful under stress, where drought, heat, nutrient deficiency, low temperature, pathogens, or excessive light can reduce photosystem II efficiency, increase energy dissipation, or alter recovery after stress (Bhagooli et al., 2021; Kalaji et al., 2016; Krause & Weis, 1991; Silva-Cancino et al., 2012). Chlorophyll fluorescence measurements are also useful for dynamic phenotyping (Kaiser et al., 2018; Murchie & Ruban, 2020). Traits such as the induction and relaxation of NPQ under fluctuating light are relevant to field crops because leaves rarely experience constant irradiance. Sunflecks, clouds, leaf movement, and canopy shading create rapidly changing light environments, and genotypes may differ in how quickly they adjust photochemistry and photoprotection (Kaiser et al., 2018; Long et al., 2022).

Chlorophyll fluorescence imaging extends point measurements to two-dimensional maps of visible plant surfaces. Imaging systems can map F_v/F_m , Φ_{PSII} , NPQ, and related parameters across leaves, whole plants, or small canopies (Chukhutsina et al., 2019; Gorbe & Calatayud, 2012; Pérez-Bueno, Pineda, & Barón, 2019; Rühle et al., 2018). This is useful because photosynthetic responses are often spatially heterogeneous. Stomatal patchiness, pathogen infection, nutrient deficiency, local photoinhibition, and senescence may produce uneven patterns that single-point measurements miss. Imaging can therefore provide not only mean values but also spatial traits such as heterogeneity, affected area, and recovery dynamics. Spatial variation in these maps can also be influenced by leaf angle, distance from the light source and camera, and partial shading by neighbouring leaves. These factors can affect illumination and signal collection, while differences in local irradiance may also induce physiological responses. Such effects should be considered when interpreting spatial patterns in chlorophyll fluorescence parameters.

Fast chlorophyll fluorescence induction, often analysed as the O-J-I-P chlorophyll fluorescence transient (OJIP), provides another rapid diagnostic tool for assessing photosystem II function, reaction centre activity, and electron transport. These traits are sensitive to stress and can be useful in screening pipelines (Kalaji et al., 2016; Stirbet et al., 2018). However, traits derived from chlorophyll fluorescence should be interpreted primarily as photochemical or stress-response phenotypes (Fu et al., 2022; Gu et al., 2019; Porcar-Castell et al., 2021; Song & Zhu, 2024). Electron transport through photosystem II can support carbon fixation, photorespiration, nitrogen assimilation, and alternative electron sinks such as the Mehler reaction (Loreto et al., 1995; Perera-Castro & Flexas, 2023; Wong & Woo, 1986). However, ETR estimates derived from chlorophyll fluorescence measurements alone cannot identify the physiological process or combination of processes responsible for an observed change in electron transport rate. These estimates should therefore be interpreted cautiously, and attributing changes to specific processes requires complementary physiological evidence. More generally, changes in ETR, Φ_{PSII} , or NPQ do not necessarily translate into proportional changes in CO₂ assimilation.

4.2. Spectral reflectance

Spectral reflectance methods measure how leaves or canopies reflect radiation at a range of different wavelengths. These signals are shaped by pigments, water, nitrogen, dry matter, internal leaf structure, surface properties, and canopy architecture (Liu et al., 2020; Mishra et al., 2017; Pandey et al., 2017). Because many of these features are linked to photosynthetic function, spectral sensing has become a major platform for high-throughput photosynthesis phenotyping. Canopy hyperspectral measurements can also support predictions of photosynthetic capacity. Multispectral approaches use wavelength bands or vegetation indices selected for their relationships with the traits of interest (Serbin et al., 2015). Examples include chlorophyll indices and SPAD-like measurements for pigment status, Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) for canopy greenness and vigour, Photochemical Reflectance Index (PRI) for xanthophyll-cycle activity and light-use efficiency, and water indices for leaf or canopy hydration (Cogliati et al., 2015; Gerhards et al., 2019; Zhang et al., 2018; Zhou et al., 2021). These indices are simple, fast, inexpensive, and easy to deploy on handheld sensors, field platforms, unmanned aerial vehicles (UAVs), or satellites (Araus & Kefauver, 2018; Xie & Yang, 2020).

Their limitation is physiological specificity. NDVI reflects canopy greenness and leaf area more strongly than photosynthetic rate per unit leaf area (Camino et al., 2019). SPAD and chlorophyll indices estimate pigment status, but chlorophyll content is not equivalent to

Rubisco capacity or CO₂ assimilation (Yendrek et al., 2017). PRI can provide information related to energy dissipation, but it is also influenced by pigment pool size, illumination, and canopy structure (Azadbakht et al., 2019; Vilfan et al., 2018). Multispectral indices are therefore useful for rapid screening and crop monitoring, but their interpretation depends on the target trait and calibration context.

Leaf-scale hyperspectral reflectance provides richer information by measuring hundreds or thousands of narrow wavelength bands. This allows prediction models to use subtle spectral features associated with leaf chlorophyll, carotenoids, nitrogen, water, dry matter, leaf mass per area, and structural traits (Mishra et al., 2017; Pandey et al., 2017; Yendrek et al., 2017). Hyperspectral data have been used to estimate leaf photosynthetic capacity traits such as V_{cmax} and J_{max} as well as associated traits such as chlorophyll content, nitrogen content, water status, and in some cases A_{net} -related traits (Heckmann et al., 2017; Robles-Zazueta et al., 2022). The advantage of hyperspectral sensing is its combination of speed and information density (Fu et al., 2022; Song & Zhu, 2024). A spectrum can be collected much faster than a gas-exchange response curve, making hyperspectral methods attractive for large genotype panels and repeated field measurements. In crop phenotyping, hyperspectral sensing can support scalable trait prediction when models are calibrated against physiological reference values, such as net CO₂ assimilation measured by gas exchange or photosynthetic capacity parameters derived from gas-exchange response curves. Furbank et al. (2021), for example, compared partial least squares regression (PLSR) with several deep-learning architectures and an ensemble model for predicting wheat leaf traits from hyperspectral reflectance. Their multi-task approach predicts multiple physiological traits jointly in a single model, exploiting correlations among traits to enhance prediction accuracy. The Wheat Physiology Predictor web tool enables researchers to apply pre-trained models to their own compatible wheat leaf hyperspectral reflectance data, making these approaches accessible for phenotyping. The resulting estimates of V_{cmax} and A_{net} represent predictions of carboxylation capacity and net CO₂ assimilation rate, respectively, and require validation against appropriate gas-exchange-derived reference values for the intended application.

At the canopy scale, spectral reflectance is more closely linked to agronomic performance because it captures leaf area, canopy closure, senescence, pigment status, and spatial variation across plots (Li et al., 2020; Xie & Yang, 2020). Canopy spectra can be collected using proximal sensors, gantries, UAVs, aircraft, or satellites. These measurements are useful for monitoring growth trajectories, stress progression, nitrogen status, and yield-related canopy development (Wang et al., 2021; Zhang et al., 2018). Canopy hyperspectral measurements also

allow estimation of photosynthetic capacity. For example, Serbin et al. (2015) used airborne hyperspectral imagery and partial least-squares regression to predict V_{cmax} and its temperature sensitivity in crop canopies. The relative importance of wavelength regions differed between the models for these two parameters. Spectral approaches are therefore best viewed as scalable predictors of biochemical, structural, and canopy-status traits, with their interpretation depending on the target trait and the reference values used for model calibration.

4.3. Thermal imaging

Thermal imaging measures leaf or canopy temperature (Lenthe et al., 2007; Pineda et al., 2020). In plants, temperature is strongly affected by transpiration because evaporative cooling lowers leaf temperature when stomata are open. When stomata close under drought, high vapour pressure deficit, salinity, or other stresses, transpiration decreases and leaf temperature often rises (Lenthe et al., 2007; Pineda et al., 2020). Thermal imaging can therefore provide information related to stomatal conductance, plant water status, and transpiration. For photosynthesis phenotyping, thermal imaging is useful because stomatal behaviour strongly affects CO₂ supply (Yi et al., 2025). A warmer canopy may indicate stomatal closure and potential stomatal limitation of photosynthesis. A cooler canopy may indicate sustained transpiration and better access to water (Blonquist, Norman, & Bugbee, 2009; Struthers et al., 2015). In drought phenotyping, canopy temperature has been widely used to identify genotypes that maintain transpiration, avoid severe water stress, or differ in water-use strategy (Casari et al., 2019; Khadka et al., 2020).

Thermal imaging is especially useful at field scale because it can measure many plots rapidly and non-destructively (Berger et al., 2010; Lenthe et al., 2007). It can also be combined with spectral or chlorophyll fluorescence measurements to distinguish water-related limitation from pigment or photochemical effects (Anagha et al., 2026; Pappula-Reddy et al., 2024). For example, a genotype with high canopy temperature and reduced assimilation may be primarily stomatally limited, whereas a genotype with normal temperature but altered chlorophyll fluorescence or spectral traits may have photochemical or biochemical limitations.

Thermal imaging should not be treated as a direct measurement of photosynthesis. Leaf temperature is affected by radiation, wind speed, humidity, boundary layer conductance, leaf angle, canopy structure, and soil background (Lenthe et al., 2007; Smigaj et al., 2023). Its relationship with photosynthesis depends on whether stomatal limitation is the dominant constraint. Thermal traits are therefore best interpreted as indicators of transpiration, canopy cooling, and stomatal behaviour, rather than as standalone estimates of carbon assimilation.

4.4. RGB, 3D, and structural imaging

RGB, 3D imaging, and related structural methods do not measure photosynthetic physiology directly, but they provide essential context for interpreting carbon gain (Al-Tamimi et al., 2022; Li et al., 2014; Liu et al., 2020). Crop carbon gain depends not only on net leaf photosynthetic capacity, but also on leaf area development, canopy architecture, light interception, phenology, senescence, and biomass allocation (Araus et al., 2021; Furbank et al., 2019; Slattery & Ort, 2021). Structural imaging can capture these features at high throughput. RGB imaging can estimate canopy cover, projected leaf area, colour, senescence, growth rate, and disease symptoms (Araus & Cairns, 2014; Mir et al., 2019; Xie & Yang, 2020). 3D imaging, light detection and ranging (LiDAR), and depth sensors can estimate plant height, canopy volume, leaf angle distribution, architecture, and lodging. These traits influence how much light a canopy intercepts and how that light is distributed among leaves (Al-Tamimi et al., 2022; Feng et al., 2021; Li et al., 2014).

This context is important because high leaf photosynthetic capacity does not necessarily produce high canopy carbon gain (Morales et al., 2020; Zhu, Long, & Ort, 2010). A genotype with strong leaf-level photosynthesis may perform poorly if leaf area develops slowly or canopy architecture limits light distribution. Conversely, a genotype with moderate leaf-level photosynthesis may accumulate more biomass through greater light interception, delayed senescence, better canopy structure, or stronger sink demand. In photosynthesis phenotyping pipelines, RGB and 3D traits should be treated as contextual traits (Fu et al., 2022; Song & Zhu, 2024). They help determine whether variation in carbon gain reflects photosynthetic capacity per unit leaf area, total photosynthetic surface area, canopy architecture, phenology, or biomass allocation.

4.5. Solar-induced chlorophyll fluorescence

Solar-induced chlorophyll fluorescence is the weak light emission from chlorophyll excited by natural sunlight. Unlike active PAM chlorophyll fluorometry, which employs artificial illumination, SIF measurements detect this emission passively using ground-based, airborne, or satellite instruments (Aasen et al., 2019; Sun et al., 2023). Because it originates from the photosynthetic apparatus and is linked to absorbed radiation and chlorophyll fluorescence yield, SIF has attracted interest as a proxy for canopy photosynthetic activity and gross primary productivity (Jonard et al., 2020; Porcar-Castell et al., 2021). For crop phenotyping, SIF offers a bridge between leaf-level chlorophyll fluorescence and canopy-scale carbon flux (Fu et al., 2022; Song & Zhu, 2024). It may be more closely related to photosynthetic activity than greenness-based vegetation indices, particularly when the goal is to monitor canopy function rather than

canopy size (Camino et al., 2019; Li et al., 2020). SIF can also capture temporal dynamics of canopy activity across days, seasons, and stress progression (Li & Xiao, 2019).

SIF is not a direct measurement of CO₂ assimilation (Gu et al., 2019; Porcar-Castell et al., 2021). The observed signal depends on absorbed photosynthetically active radiation, canopy structure, leaf angle, chlorophyll fluorescence yield, reabsorption, viewing geometry, and non-photochemical quenching (Liu et al., 2019; Sun et al., 2023). Its interpretation therefore requires radiative-transfer correction and physiological modelling (Gu et al., 2019; Liu et al., 2019). In breeding contexts, SIF may be more useful for monitoring canopy-level photosynthetic activity than for diagnosing the specific leaf-level mechanisms that underlie genotypic differences (Fu et al., 2022; Sharwood et al., 2022).

5. Toward Physiologically Grounded Photosynthesis Phenotyping

The limitation of scalable phenotyping is not that it relies on proxies, but that proxies are often used without sufficient physiological anchoring (Fu et al., 2022; Großkinsky et al., 2015; Song & Zhu, 2024). Optical and imaging methods can expand the scale, frequency, and environmental coverage of photosynthesis phenotyping (Araus et al., 2022; Fu et al., 2022). Their value, however, depends on whether the measured signal is linked to a defined target trait, validated across the intended domain of use, and interpreted within a biological framework (Sales et al., 2022). A physiologically grounded phenotyping strategy should therefore begin by defining the trait of interest, then selecting the appropriate physiological anchor and scalable readout (Figure 2). Accurate characterisation of light intensity and spectral composition, leaf and air temperature, humidity, and CO₂ concentration is essential because these conditions influence photosynthetic processes and the interpretation of physiological measurements (Busch et al., 2024). Environmental observations should accompany physiological reference measurements and scalable readouts at relevant spatial and temporal scales. These observations help interpret and calibrate relationships between sensor signals and target traits and provide inputs for mechanistic models. For example, measurements of leaf temperature, air temperature, and humidity are needed to estimate the leaf-to-air vapour pressure difference, which influences gas exchange. In this framework, reference methods define or validate specific photosynthesis-related traits, whereas high-throughput readouts extend these traits across genotypes, environments, and time. The key issue is not whether a signal is scalable, but whether its physiological interpretation matches the target trait. Future progress should therefore focus on phenotyping systems that combine directness, scalability, and interpretability.

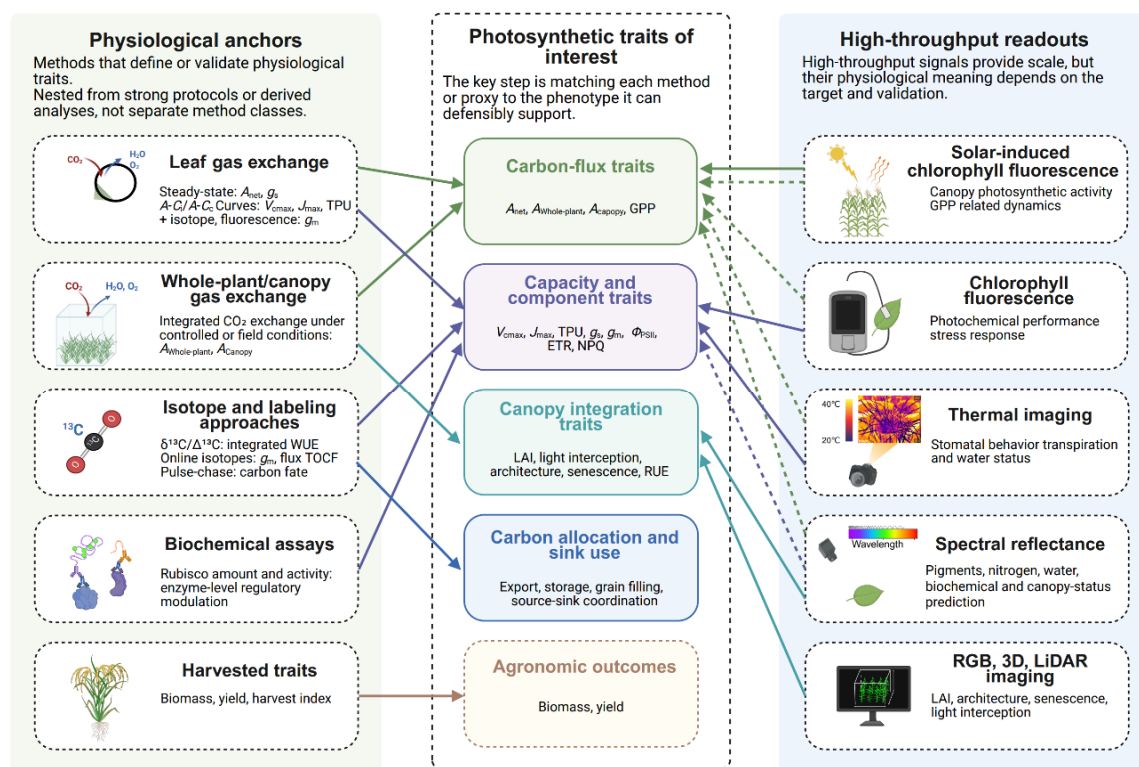


Figure 2. Linking physiological anchors, target photosynthetic traits, and high-throughput readouts. Physiological anchors on the left define or validate specific photosynthesis-related traits. The central panel groups these traits into carbon-flux traits, capacity and component traits, canopy integration traits, carbon allocation and sink use, and agronomic outcomes. The right panel lists scalable readouts, including solar-induced chlorophyll fluorescence, chlorophyll fluorescence, thermal imaging, spectral reflectance, and RGB, 3D, and light detection and ranging (LiDAR) imaging. Solid arrows indicate the primary trait class that a method or readout most directly supports or estimates, whereas dashed arrows indicate calibrated or context-dependent inference. Indirect links should not be interpreted as direct measurements of photosynthetic carbon assimilation without physiological validation. Abbreviations: $A_{\text{whole-plant}}$, whole-plant net photosynthetic assimilation rate; A_{canopy} , canopy net photosynthetic assimilation rate; C_i , chloroplastic CO₂ concentration; $\delta^{13}\text{C}$, carbon-isotope composition; $\Delta^{13}\text{C}$, carbon-isotope discrimination; TOCF, ternary-order correction factor; LAI, leaf area index. Figure created using BioRender.com.

5.1. Make reference measurements faster and more deployable

Direct and semi-direct methods remain essential because they define carbon-flux and capacity traits (Fu et al., 2022; Song & Zhu, 2024). However, conventional leaf gas exchange, CO₂ response curves, isotope labelling, and canopy flux measurements are too slow or technically demanding for routine use in large breeding populations (Du et al., 2020; Liu et al., 2025; Miao et al., 2009; Song & Zhu, 2024). A major priority is therefore to make reference measurements faster, more automated, and more compatible with field phenotyping.

Several developments are particularly important. One example is the fast photosynthesis measurement (FPM) approach developed for rice, which measures excised tillers under controlled laboratory conditions and substantially increases throughput while retaining direct gas-exchange information (Du et al., 2020). This approach can reduce environmental noise in field measurements, extend the daily measurement window, and make it feasible to screen hundreds of samples per instrument per

day, thereby improving the phenotyping of genetic variation in photosynthetic capacity (Du et al., 2020). Rapid gas-exchange protocols can also reduce the time required to estimate A_{net} , V_{cmax} , and J_{max} (Du et al., 2020), while automated or multiplexed gas-exchange systems can increase replication and capture temporal dynamics (Salter, Gilbert, & Buckley, 2018). It should be noted that while excision has only limited effects overall, the representativeness of photosynthesis-related measurements on excised leaves varies with the trait measured and the species (Ferguson et al., 2023).

Another useful direction is the development of isotope-based direct phenotyping. Although isotope and labelling approaches are unlikely to become routine screening tools for very large breeding populations, they can provide essential physiological validation (Arndt & Wanek, 2002; Liu et al., 2025; Nogués et al., 2006). For example, $^{13}\text{CO}_2$ labelling has recently been used to quantify intra-leaf photosynthetic heterogeneity during drought and rewatering, revealing spatial differences in photosynthetic recovery and water-use efficiency that are difficult to capture with conventional point gas-exchange

measurements (Liu et al., 2025). Such approaches can help validate mesophyll conductance, water-use efficiency, carbon allocation, source-sink dynamics, and spatial heterogeneity in photosynthesis (Arndt & Wanek, 2002; Liu et al., 2025; Nogués et al., 2006).

Whole-plant and canopy gas-exchange platforms can further help connect leaf physiology with crop carbon gain (Escalona et al., 2016; Kläring & Körner, 2020). These direct approaches will probably not replace optical phenotyping in very large populations (Araus et al., 2018; Fu et al., 2022; Song & Zhu, 2024). Their value lies in providing stronger physiological anchors for sensor-based phenotyping and model training (Kumagai et al., 2022; Zhou et al., 2021). As direct measurements become faster, more scalable, and better linked across leaf, plant, and canopy scales, they can improve training datasets, validate sensor models, and identify the physiological causes of genotypic variation (Du et al., 2020; Fu et al., 2022; Furbank et al., 2019; Song & Zhu, 2024).

5.2. Anchor proxies to specific physiological questions

A scalable signal should not be interpreted as “photosynthesis” in general. It should be linked to a specific question (Gu et al., 2019; Porcar-Castell et al., 2021; Song & Zhu, 2024). If the aim is to identify variation in biochemical capacity, the relevant reference traits may be V_{cmax} , J_{max} , TPU, or light-saturated assimilation (Camino et al., 2019; Kumagai et al., 2022). If the aim is drought adaptation, the relevant traits may include stomatal conductance, transpiration, canopy temperature, carbon isotope discrimination, biomass maintenance, and recovery after stress (Casari et al., 2019; Liu et al., 2025). If the aim is yield improvement, the relevant scale may shift toward seasonal canopy carbon gain, light interception, phenology, sink capacity, and harvest index (Araus et al., 2021; Xiong, 2024; Zhu, Long, & Ort, 2010).

This distinction matters because the same sensor can support different interpretations depending on the target (Cozzolino, 2023). Chlorophyll fluorescence may indicate photochemical regulation or stress injury (Krause & Weis, 1991; Murchie & Ruban, 2020; Silva-Cancino et al., 2012). Spectral reflectance may predict nitrogen, chlorophyll, water status, photosynthetic capacity, canopy cover, or senescence (Mishra et al., 2017; Xie & Yang, 2020; Yendrek et al., 2017). Thermal imaging may indicate stomatal limitation or differences in canopy energy balance (Casari et al., 2019; Pineda et al., 2020). SIF may track canopy activity, but its signal is also shaped by absorbed radiation, canopy structure, viewing geometry, and chlorophyll fluorescence reabsorption (Camino et al., 2019; Jonard et al., 2020; Porcar-Castell et al., 2021). Physiological anchoring is therefore needed to determine whether a proxy is tracking carbon assimilation, a component process, stress status, canopy structure, or downstream performance.

5.3. Validate across the intended domain of use

Proxy-model performance is often overestimated when calibration and validation are drawn from the same experiment (Kumagai et al., 2022). A model may perform well within one dataset but fail across genotypes, growth stages, environments, canopy structures, or management conditions (Cozzolino, 2023; Fu et al., 2022). This is especially important in photosynthesis phenotyping because sensor-trait relationships are context-dependent (Sales et al., 2022; Song & Zhu, 2024; Vialet-Chabrand et al., 2017). When the intended use is broad selection across years and locations, validation should include independent genotypes, developmental stages, water and nitrogen conditions, field sites, and seasons (Araus et al., 2018; Carmo-Silva et al., 2017; Sales et al., 2022).

Validation should therefore match the intended application (Sales et al., 2022). A proxy used for within-experiment ranking may not require the same transferability as a proxy used for breeding selection across environments. A model intended to estimate photosynthetic capacity should be validated against direct capacity traits, not only against biomass or yield (Camino et al., 2019; Kumagai et al., 2022). A model intended to support selection should be tested for whether it improves genotype ranking, parent selection, genetic mapping, genomic prediction, or crop-model parameterisation (Cabrera-Bosquet et al., 2012; Reynolds et al., 2020).

5.4. Move from static traits to dynamic responses

Most photosynthesis phenotyping still emphasises measurements taken at one time point or under relatively steady conditions (Murchie et al., 2018; Song & Zhu, 2024). Field photosynthesis, however, is dynamic. Leaves experience fluctuating light, temperature, humidity, water availability, and source-sink demand (Kaiser et al., 2018; Long et al., 2022). Phenotyping should therefore place greater emphasis on traits such as photosynthetic induction, stomatal kinetics, NPQ relaxation, heat response, drought progression, and recovery after stress (Kaiser et al., 2019; Taylor, 2024).

Dynamic traits may be especially important for explaining field performance (Kaiser et al., 2019; Taylor, 2024). A genotype with high steady-state photosynthesis may not have high daily carbon gain if photosynthetic induction is slow under sunflecks, stomata respond poorly to fluctuating vapour pressure deficit, or photoprotection relaxes slowly after shade (Kaiser et al., 2018; Long et al., 2022; Vialet-Chabrand et al., 2017). Conversely, a genotype with moderate steady-state capacity may perform well if it adjusts rapidly to changing light and maintains carbon uptake across the day. Dynamic protocols can therefore reveal variation that single steady-state measurements miss (Murchie et al., 2018; Vialet-Chabrand et al., 2017).

5.5. Build multi-sensor pipelines around physiological complementarity

Multi-sensor phenotyping should not mean simply adding more variables. Sensors should be combined because they capture complementary components of photosynthesis-related performance (Feng et al., 2021; Li et al., 2014). Chlorophyll fluorescence can resolve photochemical regulation (Bhagooli et al., 2021; Genty, Briantais, & Baker, 1989; Kalaji et al., 2016). Spectral reflectance can capture pigment, nitrogen, water, biochemical, and canopy-status traits (Pandey et al., 2017; Yendrek et al., 2017). Thermal imaging can indicate transpiration and stomatal behaviour (Casari et al., 2019; Pineda et al., 2020). Structural imaging can quantify canopy size, architecture, light interception, and senescence (Al-Tamimi et al., 2022; Liu et al., 2020). SIF can provide information on canopy-scale photosynthetic activity and temporal dynamics (Li et al., 2020; Porcar-Castell et al., 2021).

The value of integration lies in separating mechanisms. A reduction in carbon gain may result from stomatal closure, biochemical downregulation, photochemical stress, reduced leaf area, early senescence, poor canopy architecture, or weak sink demand (Carmo-Silva et al., 2017; Sales et al., 2021; Scafaro et al., 2023; Slattery & Ort, 2021; Souza & Yang, 2021). No single proxy can reliably distinguish all of these mechanisms. A physiologically designed sensor combination can help identify whether variation is primarily diffusional, biochemical, photochemical, structural, developmental, or sink-related (Feng et al., 2021; Fu et al., 2022; Großkinsky et al., 2015).

Mechanistic sensor-model integration can link spectral reflectance, chlorophyll fluorescence, and thermal observations to models of radiative transfer, energy balance and photosynthesis. For example, the SCOPE (Soil Canopy Observation, Photochemistry and Energy fluxes) model couples these processes to simulate canopy radiance, carbon, water, and heat fluxes (Van Der Tol et al., 2009). These frameworks provide a basis for interpreting sensor signals in relation to environmental conditions and canopy structure. Further development for routine use in photosynthesis phenotyping should focus on determining which physiological parameters can be reliably inferred, aligning the spatial and temporal scales of observations and models, and validating inferred traits against independent physiological measurements across genotypes and environments.

5.6. Connect phenotyping to breeding, genetics, and crop modelling

A photosynthesis phenotype is useful only if it supports a biological or breeding decision. For physiology, it should clarify the mechanism underlying variation (Großkinsky et al., 2015; Xiong et al., 2017;

Xiong et al., 2015a). For genetics, it should improve trait definition for mapping, association analysis, or genomic prediction (Cabrera-Bosquet et al., 2012; Flood et al., 2016; Mir et al., 2019). For breeding, it should improve genotype ranking, parent selection, or selection under target environments (Araus et al., 2018; Morales et al., 2020; Reynolds et al., 2020). For modelling, it should improve parameterisation of photosynthetic capacity, conductance, radiation-use efficiency, stress response, or source-sink coordination (Furbank et al., 2019; Heckmann et al., 2017; Yin & Struik, 2010).

The future of photosynthesis phenotyping is therefore not a choice between direct methods and proxies. Direct measurements define and validate physiological targets; scalable sensors extend those targets across populations, environments, and time (Araus et al., 2018; Fu et al., 2022; Song & Zhu, 2024). The goal is not simply to generate larger phenotyping datasets, but to generate traits that are physiologically interpretable, transferable across environments, and useful for selection, genetic analysis, or crop modelling (Araus et al., 2018; Reynolds et al., 2020).

Progress will depend on designing systems in which the target phenotype is explicit, the proxy is physiologically anchored, the validation is independent, and the final trait supports interpretation or selection. In this sense, the proxy problem is not a reason to reject scalable sensing. It is a framework for making photosynthesis phenotyping more reliable, transferable, and useful for crop improvement (Araus et al., 2018; Song & Zhu, 2024).

6. Conclusions

Photosynthesis phenotyping is essential for crop improvement, but its main challenge is not only technical throughput. It also lies in defining and interpreting the phenotype being measured. Photosynthesis may refer to carbon assimilation, biochemical capacity, photochemical regulation, CO₂ diffusion, canopy carbon gain, biomass, or yield. These traits are related, but they are not interchangeable. A useful phenotyping method must therefore be evaluated against a clearly defined target phenotype. Direct and semi-direct methods, including gas exchange, isotope-based approaches, and canopy flux measurements, provide the physiological reference traits needed to define and validate photosynthetic phenotypes. Scalable optical and imaging methods provide the speed, spatial coverage, and temporal resolution needed for large populations and field environments. These two groups of methods should therefore be viewed as complementary. Reference methods anchor physiological meaning, whereas scalable methods extend measurement across genotypes, environments, and time. Future progress will depend on moving from proxy collection to physiologically grounded phenotyping. This requires

explicit target traits, appropriate reference measurements, independent validation, dynamic protocols, and multi-sensor integration. The goal is not simply to measure photosynthesis faster, but to generate traits that explain physiological variation, improve genotype ranking, support genetic analysis, and contribute to crop modelling and breeding decisions.

Author Contributions

D.X. developed the initial concept and W.C., N.L. and D.X. wrote the manuscript. All authors contributed to writing, editing, and figure preparation. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. Given the role as Associate Editor, Dongliang Xiong had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used Doubao (ByteDance) to a limited extent to assist with language editing, primarily focusing on word choice, sentence structure, and improvements in clarity and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Peer Review Statement

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