in silico
Plants
OXFORD

Opinion

Carbon storage in plants: a buffer for temporal light and temperature fluctuations

Ana Cristina Zepeda*[iD], Ep Heuvelink and Leo F. M. Marcelis*[iD]

[1]Horticulture and Product Physiology, Department of Plant Sciences, Wageningen University, PO Box 16, 6700 AA Wageningen, The Netherlands

*Corresponding authors' e-mail addresses: cristina.zepeda@wur.nl; leo.marcelis@wur.nl

ABSTRACT

Carbon (C) storage allows a plant to support growth whenever there is a temporal asynchrony between supply (source strength) and demand of carbon (sink strength). This asynchrony is strongly influenced by changes in light and temperature. In most crop models, C storage is included as a passive process that occurs whenever there is an excess of C from photosynthesis compared with the demand of C for metabolism. However, there are numerous studies that challenged this concept, and provided experimental evidence that C storage is an active process that allows buffering of environmental fluctuations and supports long-term plant growth. We propose that an active C pool needs to be included in simulation models for a better understanding of plant growth patterns under fluctuating environment. Specifically, we propose that the two main mechanisms actively regulating C storage in plants are the partitioning of assimilates between soluble sugars and starch and the degradation and remobilization of storage compounds. The insights gained here are important to optimize crop performance under fluctuating conditions and thus for developing more resource-efficient crop production systems.

KEYWORDS: Carbon metabolism; carbon storage; crop growth modelling; light; source–sink interaction; temperature.

1. INTRODUCTION

Storage is a fundamental process where plants build up resources that can be mobilized under future and less favourable conditions to support biosynthesis for growth or other plant functions (Chapin *et al.* 1990). Carbon (C) storage buffers temporal asynchrony between C supply by photosynthesis and C demand by the different plant organs. Plant carbon metabolism is often described as a relationship between autotrophic organs acting as 'source' (net producers of carbon) and heterotrophic organs acting as 'sinks' (net importers of carbon) (MacNeill *et al.* 2017). Within this context, the C status of the plant can be either source-limited or sink-limited. In source-limited plants, the net production of assimilates is less than the net demand of assimilates; therefore, plant growth is limited by C supply (Smith and Stitt 2007). On the contrary, in sink-limited plants, the net production of assimilates exceeds the demand and growth is limited by assimilate usage (Palacio *et al.* 2014).

In nature, plants need to constantly cope with a rapidly changing environment. Plants are subject to diurnal variations in light,

as the natural course of radiation during the day follows a sinusoidal pattern but temperature also changes diurnally, with higher temperatures during the light period and lower temperatures at night. Additionally, plants needs to cope with unpredictable environmental fluctuations on a day-to-day basis, as natural light, ambient temperature and water availability vary depending on the weather on a particular day and because of slower alterations due to seasonal effects (Parent *et al.* 2010). These fluctuations have a profound effect on carbon fluxes such as photosynthesis, respiration, growth and storage (Fatichi *et al.* 2014; Gessler and Grossiord 2019). For example, during the light period, carbon is fixated by photosynthesis which is highly dependent on light (Farquhar *et al.* 1980) while respiration is mainly dependent on temperature. During the dark period, only mitochondrial respiration and growth take place and these processes are highly influenced by temperature and substrate availability. Upon a sudden increase in light intensity, photosynthesis is limited by enzyme activation in the Calvin–Benson cycle (Kaiser *et al.* 2018) or by energy dissipation (Kromdijk *et al.* 2016). Temperature, on the

other hand, influences several metabolic processes that determine cell expansion and cell division, and thus the growth rate of organs (Parent *et al.* 2010).

The source–sink balance accounts for asynchronies between the supply of assimilates from photosynthesis and the demand of assimilates for respiration and growth. Within this context, the build-up of C storage through time is interpreted as a passive process that occurs only when C supply exceeds C demand by different sinks (Chapin *et al.* 1990). This interpretation, however, has been challenged several times in the past (e.g. Cannell and Dewar 1994; Sala *et al.* 2012; Dietze *et al.* 2014) and it is argued that C reserves cannot be treated as passive reservoirs or as an 'optional extra' (Da Silva *et al.* 2014). Instead, a more accurate view is that C storage is a constantly active process, where C storage is a sink that competes for carbohydrates to a greater or a leaser extend in specific instances, according to environmental conditions (Chapin *et al.* 1990; Dietze *et al.* 2014). Active storage, therefore, involves metabolically regulated partitioning or synthesis of C to storage compounds from resources that would otherwise be used for growth (Gessler and Grossiord 2019).

Despite the long list of studies on source–sink interactions (e.g. Sonnewald and Fernie 2018), there is still a widely debated question: is C assimilation or rather C usage ultimately responsible for crop yield? There is a strong line of research that argues that C assimilation (source activity) ranks above any other process that drives plant growth (De Souza *et al.* 2017) while there are others that argue that both source and sink effects co-limit plant growth (Fatichi *et al.* 2014; Körner 2015). For plants to grow they need resources and appropriate conditions so that these resources are converted into biomass. When conditions are temporarily unfavourable, resources are stored temporarily, and in this sense the C storage process becomes also an important process as well, although only partly understood (Dietze *et al.* 2014; Hartmann and Trumbore 2016).

Although C has a central role in plants, there is no clear understanding about how allocation of C to storage and remobilization of C occurs in response to temporal environmental stresses. Moreover, an active C storage is rarely explicitly included in crop growth models (Dietze *et al.* 2014). Here, we propose that crop growth models should include an 'active' storage compartment based on biochemical processes in order to quantify plant responses to fluctuations in light and temperature. Incorporating a dynamic active C storage pool would allow understanding plant strategies to cope with fluctuating environments as an integrated process: from fluctuations in environmental conditions to C-pool dynamics to growth of plants. We first discuss experimental evidence of the physiological mechanisms that drive C storage in plants. Specifically, we propose that the two main mechanisms actively regulating C storage in plants are the partitioning of assimilates between soluble sugars and starch and the degradation and remobilization of storage compounds. Second, we discuss the possibility of extrapolating insights gained from diel storage regulation to the long-term (days to weeks) dynamics of C storage and its consequences for growth. Finally, we propose a modelling framework to simulate storage accumulation and remobilization based on biochemical processes. The insights gained here are important to optimize crop performance under fluctuating conditions and thus for developing more resource-efficient crop production systems.

2. THE ACTIVE REGULATION OF C STORAGE

We identify two mechanisms as key for the active regulation of C storage: (i) partitioning of assimilates between soluble sugars and starch and (ii) degradation and remobilization of storage compounds. Carbon is assimilated by plants via photosynthetic uptake of atmospheric CO_2, producing C-rich compounds such as glucose, fructose, sucrose, starch but in some herbs and grasses also fructans, oligosaccharides, polysaccharides other than starch (e.g. inuline), all referred to as non-structural carbon (NSC) (Martínez-Vilalta *et al.* 2016). These are then partitioned among different processes such as respiration, structural growth, reproduction, storage and defence (Chapin *et al.* 1990). Structural carbon is fixed in plant structure such as cell walls, composed of cellulose, hemicellulose or lignin. These compounds cannot be re-used by the plant in the future because plants lack the enzymes for cellulose degradation (Furze *et al.* 2018). Non-structural carbon (NSC), on the other hand, functions as a source of stored energy and carbon for biosynthesis (Dietze *et al.* 2014). Sucrose synthesized during the day is either converted to starch or used to satisfy immediate respiratory requirements or is exported from the leaves to sink organs such as young leaves, reproductive organs or fruits (Ruan 2014; MacNeill *et al.* 2017). Other roles of sucrose are for cell osmoregulation (Talbott and Zeiger 1998), as a signalling molecule to regulate gene expression, for crosstalk with hormonal, oxidative and defence signalling (Ruan 2014) and as a transport sugar (Hartmann and Trumbore 2016). In contrast, starch is the most prevalent form of long-term carbohydrate reserve in trees (Da Silva *et al.* 2014), and in herbaceous plants can be transiently stored to fulfil C demand during the night (Thalmann and Santelia 2017) or for later use in periods of low C supply like winter in perennial plants.

2.1 Assimilate partitioning: between sucrose and starch

One key process part of the active regulation of C storage is the partitioning of newly assimilated carbon between soluble sugars and starch during the day (Smith and Stitt 2007). This is considered as an active response because assimilates are not immediately used, but rather partitioned into sucrose for the immediate demands during the day, but also partitioned into starch to fulfil C demand during the following night (Graf *et al.* 2010a; Graf and Smith 2011; Stitt and Zeeman 2012). This mechanism is nicely demonstrated in starchless *Arabidopsis pgm* mutants which have much lower growth rates than the wild-type plants when grown at low light intensities or short days; however, their growth is normal in continuous light or very long days (Gibon *et al.* 2009; Graf *et al.* 2010a). Partitioning of assimilates to starch is therefore strongly dependent on photoperiod, but weakly dependent on light intensity. For example, growing *Arabidopsis* in an 8-h photoperiod at 28 °C showed that when light intensity was reduced from 160 to 40 µmol m^{-2} s^{-1} for one photoperiod, relative allocation fraction to starch was not changed (Pilkington *et al.* 2015). A similar response was also observed by Mengin *et al.* (2017) as they showed that halving the duration of the light period (at a given light intensity) led to an increase of 41–61 % in the fraction of assimilates allocated to starch, while halving the light intensity at a given photoperiod led to an increase of the relative partition to starch of only 7 %. A higher partitioning into leaf starch was observed in different species such as corn,

soybean, spinach and sugar beet when plants were shifted from a long to a short photoperiod (Chatterton and Silvius 1980).

Evidence of an active partitioning of C between immediate growth and storage has been also demonstrated in deciduous trees. Silpi *et al.* (2007) showed that, by tapping rubber trees to deplete the C reserves during the growing period (i.e. summer), the rate of C accumulation to storage increased to compensate for the depletion of the reserves caused by the latex production. They concluded that C storage appears to be an active sink that could compete with growth even in moments when growth rates are high. Similarly, although autumn is the main period for C accumulation in temperate trees, significant amounts of C were accumulated during active growth in the summer period (Barbaroux and Bréda 2002; Landhäusser and Lieffers 2003).

Assimilate partitioning between soluble sugars and starch at the gene and enzyme levels occur in response to sugars and is mediated by light and clock signalling (Stitt and Zeeman 2012). In the past, starch synthesis in leaves was explained by a simple overflow product synthesized when the rate of CO_2 fixation exceeds the rate of sucrose synthesis (see MacRae and Lunn 2006 for detailed mechanism). Although this mechanism indeed occurs in plants, this model cannot explain how starch accumulation is regulated to guarantee an adequate supply of C at night in source-limited plants. An alternative (or complementary) mechanism is that C partitioning is programmed so that a fixed fraction of assimilates is allocated for starch synthesis. This occurs mediated by a central enzyme for starch synthesis, AGPase, which is redox-regulated by a light-dependent signal leading to changes in trehalose-6-phosphate (T6P) levels (Kolbe *et al.* 2005). These changes in sucrose levels give feedback to the circadian clock, which in turn synchronizes circadian oscillators that affect the expression of the circadian genes allowing the plant to adapt to changes in day length (Edwards *et al.* 2010; Feugier *et al.* 2013; Seki *et al.* 2017). Therefore, starch synthesis involves a complex interaction of regulatory mechanisms rather than only an overflow, although starch still can be synthesized via an overflow when sucrose levels are too high (Rasse and Tocquin 2006).

Including this flexible partitioning of newly assimilated C between soluble sugars (for immediate use) and starch (for storage purposes) would be a first step towards representing the C storage pool in plants as an 'active' process. However, predicting partitioning quantitatively across different environmental fluctuations and/or across different species is still challenging. Assimilate partitioning may differ between species, according to their form of C storage (i.e. sucrose-accumulating species such as wheat and rice, starch-accumulating species such as *Arabidopsis* or tomato, or species that accumulate both such as maize or sorghum) (Liang *et al.* 2021). These differences can lead to alternative C balance strategies under changing environments among different species, which ultimately affects growth. For instance, Wiese-Klinkenberg *et al.* (2010) showed that in the monocotyledon *Zea mays*, diel leaf elongation patterns followed changes in temperature. Contrary, in the dicotyledon *Nicotiana tabacum*, the effect of different temperatures is less obvious and leaf elongation followed a clear circadian oscillation. In deciduous trees, temperature plays a key role in the carbohydrate metabolism, as energetic reserves are a requisite for trees to bloom. Dormant trees regulate structural carbon concentrations during winter through an increase in the starch synthase pathway when

temperature is cold. Later on, when temperatures rises, starch synthase pathway is decreased and starch degradation pathway is promoted (Sperling *et al.* 2019).

2.2 Remobilization of storage

The second key process to explain the active regulation of storage in plants is the degradation of starch into sucrose at night, or in the long-term, the remobilization of previously stored NSC. On a diurnal basis, the starch degradation rate is set by a mechanism that senses the amount of starch in the leaves at the end of the day, and anticipate the length of the night (Smith and Stitt 2007). While the mechanisms in which the plant senses the amount of starch at the end of the day are not fully elucidated yet (although see Smith and Zeeman 2020), there is evidence that the anticipation of the night is tightly coordinated with the circadian clock and sucrose sensing (Gibon *et al.* 2004, 2009; Scialdone *et al.* 2013; Pilkington *et al.* 2015). When plants are grown under very low light intensities or short day lengths, the stored starch during the day is almost completely remobilized precisely by dawn. Under these circumstances, plants can quickly adjust the rate of starch degradation to an unexpected shortening of the light period, as long as the 24-h periodicity of a diel cycle is kept. If the diel cycle is shorter or longer than 24 h, the circadian clock cannot predict the change in periodicity and adjusts the rate on a 24-h basis which results in incomplete or too rapid degradation (Graf *et al.* 2010b).

An important observation here is that this 'near-to-complete' degradation of storage has been shown almost only in *Arabidopsis* plants grown under source-limiting conditions (light intensities between 90 and 170 μmol m^{-2} s^{-1}) (Gibon *et al.* 2004, 2009; Scialdone *et al.* 2013; Sulpice *et al.* 2014; Pilkington *et al.* 2015). There is very limited research done regarding starch degradation at night in plants grown at light intensities that are not limiting photosynthesis during the day. One of the few exceptions is work from Pilkington *et al.* (2015), where they compared starch degradation patterns between plants grown under low light intensities (140 μmol m^{-2} s^{-1}) and higher light intensities conditions (240 μmol m^{-2} s^{-1}). They concluded that under sink-limiting conditions, there is incomplete remobilization of starch at night, and that the rate of degradation of starch into sucrose can be then increased by increasing the temperature. These conclusions are very relevant for plants grown under in the field, as usually plants are exposed to higher light intensities, at least during spring and summer, or at the middle of the day. The incomplete remobilization of starch at night can lead to two situations. First, below a 'saturation' threshold, incomplete remobilization of starch can lead to a build of C reserves for plants to sudden changes in C availability or sudden changes in the night temperatures. Second, the constant increase of starch content from day to day above a saturation threshold can ultimately cause feedback inhibition of starch breakdown by the sucrose signal T6P (Camara *et al.* 2013) and to a decrease in the expression of photosynthetic genes causing a downregulation in photosynthesis (Paul and Foyer 2001). Both situations can have an effect at a whole-plant level and productivity.

In addition to the diurnal degradation of starch to soluble sugars in leaves to support growth, remobilization of stored NSC to the grains is maintained through the diurnal cycle in the grain-filling stage in wheat and barley (Fisher and Gifford

1986; Schnyder 1993). Temporary NSC storage pools also are involved in supplying C to the grains during periods of fluctuating concurrent photosynthesis or when current photosynthesis is low at the beginning and end of the natural light period (Geiger *et al.* 2000).

To make progress in the prediction of diel starch remobilization in crops, it is necessary to have a better quantification of the starch usage at night under sink-limited conditions and a better quantification on the effect of temperature on the rate of degradation. Additionally, starch remobilization must be studied over a wider range of species, ideally with different metabolisms (i.e. C_3 and C_4 plants), with different life cycles (i.e. annual and perennial) and different responses to day length (short-day, neutral-day, and long-day plants). Finally, the fine limits between when do plants switch from source-limited conditions and sink-limited conditions, and when they are 'starch-saturated' is still needed.

2.3 Long-term accumulation and remobilization of storage

Although it is true that the circadian rhythm is in charge of the remobilization of storage on a diel basis, a remaining question is: how are NSC reserves remobilized in the longer term, for example, on a seasonal basis? Carbon storage in other organs such as roots becomes crucial, for example, in trees or in geophytes to resprout after winter (Clarke *et al.* 2013; Wiley *et al.* 2019), and in stems and other vegetative tissue in cereals (mostly as fructans) to satisfy their energy requirements during the annual life cycle (Pollock and Cairns 1991; Pommerrenig *et al.* 2018). These storage pools are often considered as 'long-term' rather than a 'readily available' storage pools because they act as sinks that store energy reserves in winter when aerial growth stops. Later, in spring, they become a source and remobilize the stored C to subsequent filling of the grain or the formation of new tillers or resprouting (Pollock and Cairns 1991; Goudriaan and van Laar 1992; Boscutti *et al.* 2018).

An interesting example that highlights the importance of seasonal C reserve pool and accumulation–depletion of NSC reserves on grasses is during the grain-filling period. Two types of sources contribute with carbohydrates for grain filling: current photosynthesis but also the remobilization of NSC stored in vegetative tissue before the onset of grain filling (Serrago *et al.* 2013). Non-structural carbon reserve pools provide the substrate needed to maintain transport and the supply of assimilates to grains during the dark period of the diurnal cycle, but also during the latter part of grain filling when growth rate of the ear is at its maximum (i.e. 3 weeks after anthesis) (Schnyder 1993). Therefore, cereals have the capacity to compensate for reductions in source–sink ratios based on the stored assimilates on the stem.

In addition to seasonal storage and remobilization of NSC, when plants are exposed to continuous environmental stress, C partitioning towards storage can increase, at the cost of the usage of C for immediate growth. For example, Huang *et al.* (2018) observed that in spruce trees grown under source limitations (120 ppm CO_2) growth and respiration were downregulated in a manner that maintained NSC concentration levels that were necessary to prevent C starvation ('operational NSC levels'). Using isotope labelling Hartmann *et al.* (2015) showed that C was partitioned to storage pools independently of the net flux and even under severe C limitation. Similarly, in a study using

isotope labelling, mint plants grown under low CO_2 and water deficit showed that newly assimilated carbon was used for monoterpenes production, a metabolite related to defence (Huang *et al.* 2019). These examples illustrate how in trees and herbaceous plants exposed to long-term abiotic stress, C is actively partitioned into storage and secondary metabolites at the expense of growth, which results in a reduced risk of starvation and can provide defence molecules to protect plant tissues.

From these examples we can conclude C allocation and remobilization in plants are continually changing in response to the C status of the plant, which is in turn highly dependent on the environment. In reality, most likely there are multiple C pools with different mean resident times (Dietze *et al.* 2014) and this represents a challenge for the characterization of the crop as a system.

2.4 The signalling role of carbohydrates in the regulation of plant growth

Plant growth depends largely on the availability of C as a substrate; however, carbohydrates can also drive plant growth acting as signalling molecules that interact with environmental cues that coordinate cell growth with storage and nutrient remobilization (L. Li and Sheen 2016). For example, the conserved protein kinases SnRK1 (Snf1-related protein kinase 1) and TOR (target of rapamycin) are energy-/sugar-sensing molecules that have an essential role in the regulation of metabolism and gene expression of plants under unfavourable environmental conditions (Margalha *et al.* 2019; Rodriguez *et al.* 2019). SnRK1 is activated in response to declining energy supplies (e.g. unexpected darkness or extended night, herbicide feeding or hypoxia) (Baena-González *et al.* 2007) triggering the activation of catabolism and repressing energy consuming anabolic processes and growth (Baena-González and Hanson 2017). An increase in the levels of sucrose (Suc) increases the signalling sugar T6P inhibiting the activity of SnRK1, therefore stimulating growth of cells and their metabolic activity (Zhang *et al.* 2009; Nunes *et al.* 2013; Lawlor and Paul 2014). Conversely, TOR kinase is activated in favourable energy conditions (e.g. by light, sugars and inorganic nutrients) to promote growth and downregulated under stress conditions that restrict sugar availability (Rodriguez *et al.* 2019). Thus, plant energy/sugar also influences plant growth via activation or repression of SnRK1/TOR.

Growth depends also in the rate of protein synthesis, which occurs in the ribosomes (Lastdrager *et al.* 2014). It has been shown that ribosome abundance does not change between different photoperiods, but rather the plant optimizes for a better distribution of protein synthesis over a 24-h cycle (Sulpice *et al.* 2014). This has an impact in the distribution of growth (as cell expansion) between day and night.

3. MODELLING THE CARBON POOL UNDER LIGHT AND TEMPERATURE FLUCTUATIONS: SHOULD AN ACTIVE C STORAGE POOL BE INCLUDED?

Quantifying changes in the C storage pool in response to light and temperature fluctuations at a whole-plant level can be challenging due to the complexity of the interactions between circadian signals, environmental cues and metabolic signals. There is also complexity because light primarily affects source activity,

while temperature primarily affects sink activity (Fatichi *et al.* 2014). Plants use most of the newly assimilated C for growth and respiration. However, under environmental stress, recently fixed C is allocated towards reserve formation at the cost of short-term growth (Dietze *et al.* 2014; Mengin *et al.* 2017; Gessler and Grossiord 2019; Huang *et al.* 2019). This entails complex feedback in which plants sense C storage level and according to day length and carbon status, plants induce changes in the partitioning and use of C, which in the end affects the growth pattern of the plant. Unravelling these dynamics requires a modelling approach that can accurately describe C storage metabolism and link it to plant growth.

There are still knowledge gaps that represent a challenge for model development. For example, it is still unclear what the metabolic costs of C storage are, which fraction of newly fixed C is partitioned to storage and which fraction is immediately used, what is the sink strength of C storage itself and implications of C storage over a long-term for herbaceous species or fruiting crops. In this section, we briefly review the way in which C storage is currently included in crop growth models and we discuss the role of the C storage pool affecting the response of the modelled system.

3.1 Approaches to simulate a dynamic storage C pool

Most crop production models are driven by the effect of shoot environment on gross photosynthesis and respiration over a day (Poorter *et al.* 2013). Growth over time is modelled as the net result of daily C input from gross photosynthesis minus C loss in respiration. However, this approach overlooks that in moments when supply exceeds demand, C should be temporarily stored. A more accurate analysis of gains and losses of C over the diel cycle should consider the asynchrony between the timing of acquisition of C and of the utilization of C for growth and respiration. At each time step, all C input from photosynthesis is directly placed in a temporary storage pool (NSC) and partitioned among different pools based on allometry, functional equilibrium or relative sink strengths of the plant organs (Marcelis *et al.* 1998). Allocation to storage occurs only if there is an excess of C from supply compared to demand. Although these growth models have been developed a long time ago (e.g. Thornley 1976; Marcelis *et al.* 1998), they are still commonly used to model C source–sink relations (e.g. Vanthoor *et al.* 2011; Seginer and Gent 2014; Cerasuolo *et al.* 2016; Gu *et al.* 2018). The following three examples illustrate how a dynamic C storage pool was included in growth models. In each example, the storage pool is defined using different approaches.

In the first example, Da Silva *et al.* (2014) updated the L-PEACH model to simulate annual long-term carbohydrate storage and mobilization. They modelled the sink strength of storage as a function of the total NSC content. Storage sink strength was 0 as long as NSC were below the minimum value. Then, storage sink strength increased along a logistic curve to reach 1 (so maximum sink strength capacity) when NSC levels reached the maxim level. In this way, storage sink strength depended on the local carbohydrate conditions, and thus, NSC concentration patterns appeared as an emergent property of the model. This approach requires the storage sink strength and the maximum carbohydrate available for remobilization to be experimentally determined. Da Silva *et al.* (2014) determined the potential storage sink strength from the mean maximum trunk NSC mass fractions. The carbohydrate storage source available for remobilization was estimated from the difference between the maximum mass fraction of carbohydrates in the sapwood and the minimum mass fraction under 'healthy' conditions (no water stress, severe pruning or disease). With this approach, annual carbohydrate storage behaviour in trees was simulated accurately.

In a second example, Jing *et al.* (2020) used a crop growth model to systematically analyse the response of perennial alfalfa under fluctuating environmental conditions and to evaluate potential adaptation options. In their model, C remobilization from storage organs to sinks was included as a function of NSC content, nitrogen content and the residual leaf area after harvest in perennial forage. Mobilization was accelerated under low leaf area index (LAI) until plants had sufficient leaves for maximum photosynthesis; only until then, C starts accumulating again.

In the third example, starch accumulation in wheat grains was modelled by focusing on the variations of plant carbon dynamics between pre- and post-anthesis stages. Assimilates for starch synthesis during grain filling can be obtained via remobilization of stored C in vegetative tissues during pre-anthesis stage or from ongoing photosynthesis (Schnyder 1993). Pan *et al.* (2007) modelled the NSC dynamics by separating the C source between an instant pool (i.e. instantaneous translocation of assimilates to wheat grains), and a long-term storage pool (i.e. remobilization of pre-stored C reserves). The remobilization of the stored NSC was influenced by temperature, water and nitrogen conditions. Additionally, they included a genetic parameter to describe the difference in starch synthesis ability among cultivars. This model was particularly useful to quantify grain starch concentration and starch yield of wheat under various growing conditions.

3.2 Sucrose–starch dynamic models

As C storage ultimately depends on the rates of synthesis and degradation of starch, one way forward is to include the biochemical regulation of sugar–starch dynamics in growth models. Starch degradation has been described by two different models: arithmetic division (AD) and retrograde metabolic signalling (RMS) models (Smith and Zeeman 2020). The AD model accounts for the rapid adjustment of the degradation rate to unexpected changes in the length of the night. In this model, the plant senses starch content and time until dawn and sets a rate of starch degradation that will deplete reserves at dawn (Scialdone *et al.* 2013). The RMS model assumes that a plant needs to maintain carbon homeostasis. The plant regulates the expression of the circadian clock genes via sugar signalling and adjusts the phase of the clock accordingly (Feugier *et al.* 2013; Seki *et al.* 2017; Webb *et al.* 2019). The main difference between these two models is that the RMS model accounts for mechanisms that control an integrated response between starch synthesis and degradation so that sugar homeostasis is maintained while the AD model simply sense starch content and time remaining until dawn.

There are a few models simulating crop growth that include sucrose synthesis and starch degradation. For example, Rasse and Tocquin (2006) used single-rate modification of starch

synthesis and breakdown in leaves to predict different growth patterns between wild-type and mutants of *Arabidopsis* at a high CO_2 concentration. Flis *et al.* (2015) adopted a more complex approach by using integrated multiscale models that link gene expression dynamics, carbon partitioning to organ growth and development in response to environmental signals. In their model, however, starch mobilization was underestimated in long photoperiods. This might be attributed to the fact that in this case the plants were probably sink-limited (Poorter *et al.* 2013). Another limitation is that in both crop growth models (Flis *et al.* 2015 and Rasse and Tocquin 2006) a fixed ratio between sucrose synthesis and starch accumulation was assumed. This ratio was empirically parameterized based on measurements in controlled growth conditions. A fixed ratio between sucrose synthesis and starch accumulation assumes that different day lengths have no effect on C partitioning, which prevents the model to adapt to changes in day length.

3.3 Simulating the effect of temperature and light fluctuations on growth

A pitfall in crop growth models to predict growth in a fluctuating environment is that they are mostly source-driven (Marcelis *et al.* 1998), meaning that photosynthesis is the rate-controlling factor for plant growth. Not surprisingly, the Calvin–Benson cycle has been the most intensively modelled biochemical process. The development of a well-calibrated and complete biochemical mathematical description of photosynthesis at leaf level has made it possible to obtain accurate predictions of photosynthesis under a wide range of conditions (Yin and Struik 2009). A source-driven approach seems logical in highly controlled environments where no water, temperature or nutrient limitations occur. However, growth can also be limited by sink activity, for example, at low temperature or high CO_2 concentrations. In these cases there is a restriction in growth together with an accumulation of C (Körner 2015).

Another limitation to accurately predict growth rate diurnally or in the short term (hourly) is that many plant growth models restrict growth only to the light periods and do not include knowledge on how C is used for metabolism and growth at night (e.g. Thornley 1976; Henten 1994; Vanthoor *et al.* 2011). When the C pool has no distinction between sucrose and starch, it is assumed that all carbohydrates are used immediately for daytime growth, or in case of an excess, stored transiently for the next light period. However, during the dark period, C is still being used for maintenance respiration and if the C pool is empty at the end of the light period (due to source-limited conditions), this can virtually lead to 'negative' growth at night. This raises two issues, first that without the distinction between sucrose and starch pools, it is unlikely to reproduce the critical mechanisms that drive the C cycle on an hourly basis. Second, it prevents understanding the effect of environmental fluctuations on growth in the day separately from the night. For example, if the goal is to study the effect of night temperature on night growth, with current models it is only possible to interpret the results as the effect of an average daily temperature or a temperature difference, rather than the effect of the night temperature itself on growth. Contrary, if the interest is only on the dynamics over a whole-day basis, the diurnal dynamics between soluble sugars and starch become less relevant.

3.4 Modelling growth with or without a C pool

In order to illustrate our argument that modelled growth patterns will be more realistic by including a C storage with the concepts discussed in the previous sections, we compared three models that differ only in the inclusion of a C pool. The first model features a static C allocation scheme and has no C storage pool. At each time step, a fixed proportion of the current assimilates is immediately lost through respiration and the remainder is allocated to growth [**see Supporting Information—Eq. S1**].

In the second model [**see Supporting Information—Eqs. S9 and S10**], C is first allocated to a dynamic storage pool from which it may be allocated to structural growth. The storage pool builds up when current assimilate production is greater than C allocation to respiration and structural growth and is depleted when the production of assimilates is smaller. This model divides respiration into growth and maintenance components. Growth respiration is proportional to structural growth and maintenance respiration is temperature-sensitive and proportional to biomass. In this model, relative growth rate [**see Supporting Information—Eq. S12**] depends on the concentration of NSC (Thornley and Hurd 1974).

In the third model [**see Supporting Information—Eqs. S15–S17**] there are two C pools: one for soluble sugars and one for starch. All assimilates produced by photosynthesis are partitioned between starch and soluble sugars depending on the photoperiod (Pilkington *et al.* 2015; Mengin *et al.* 2017). All assimilates allocated to the soluble sugar pool are used for structural growth and respiration. During the day the starch pool builds up and it is not used. During the night, the accumulated starch is degraded into soluble sugars at a constant rate according to the length of the night (Scialdone *et al.* 2013) until it is almost depleted at the end of the night (Gibon *et al.* 2004; Graf *et al.* 2010b). To avoid size-induced differences, in the models we considered a fixed crop size of 200 g m^{-2} and a leaf area index of 3. We compared the accumulated structural growth between the three models and the dynamics of the pools (when a pool is present). Detailed information about the models is provided in **Supporting Information**.

As outlined in Section 2, changes in light and temperature lead to temporal asynchronies between C supply by photosynthesis and C demand for growth. The source–sink ratio can be manipulated by light and temperature conditions. Following this logic, we initialize our simulation assuming a balanced source–sink ratio for 10 days (therefore, there is no accumulation or remobilization of C during this period) (Fig. 1A). After 10 days, as light intensity increases and temperature decreases, the C supply is bigger than the demand, and the NSC pool starts building up in both models that include a C storage pool (Fig. 1A). A similar dynamic pattern of the NSC has been observed previously under similar environmental conditions (Klopotek and Kläring 2014). The main difference between the three models is that during this period of 'accumulation', the models with a C pool partitioned recently fixed C towards storage formation at the cost of short-term growth of structural mass (Fig. 1A and B), compared to the model without C pool that has a higher growth because all assimilates are immediately used for growth. In both models with storage the stored C is used later to give a higher growth rate for a few days once C supply becomes more limiting (Fig. 1C) (a high temperature and a low light intensity, from Day
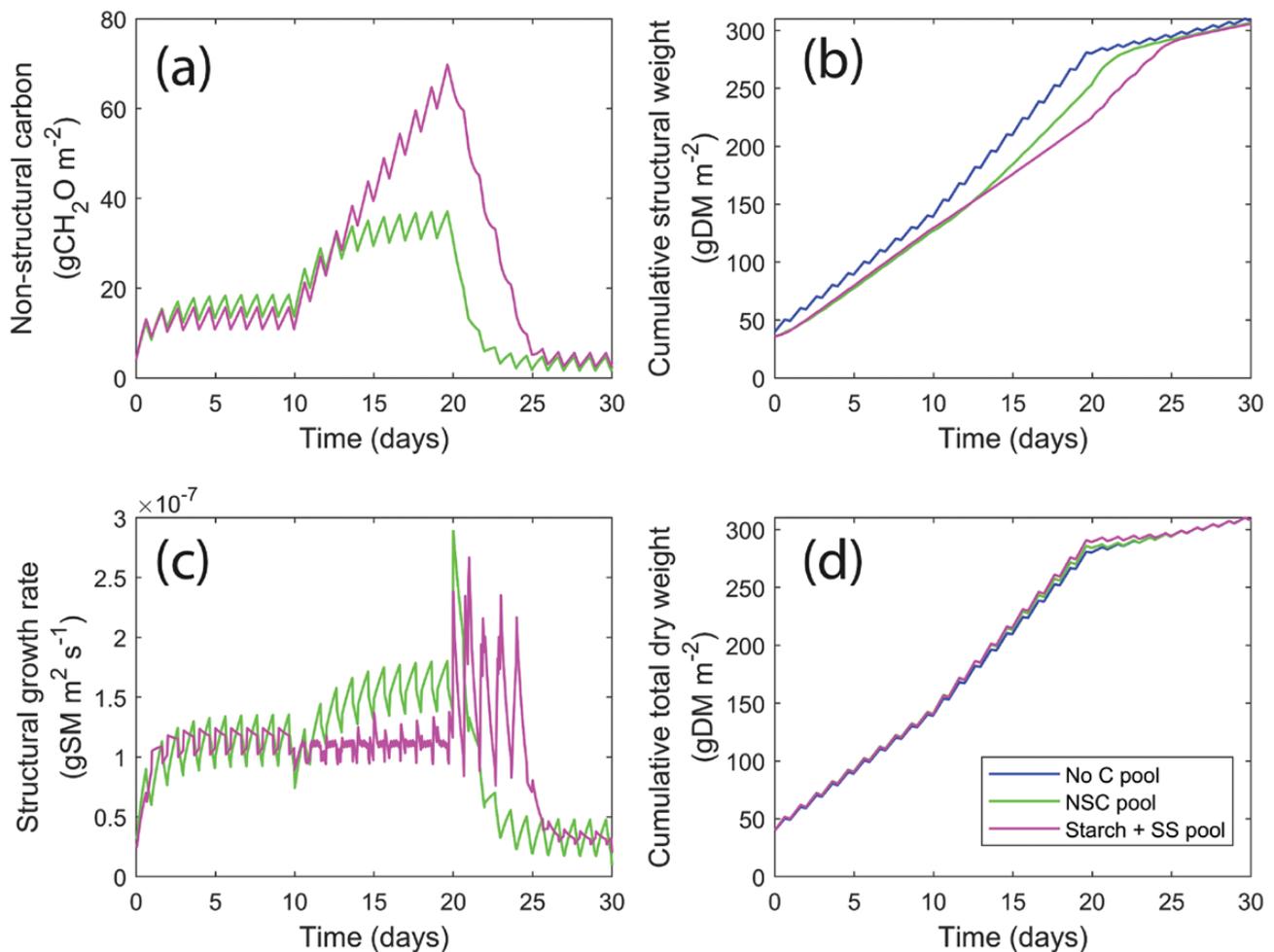
Figure 1. Simulated (A) non-structural carbon (soluble sugars + starch), (B) cumulative structural mass, (C) structural growth rate and (D) cumulative total dry weight. Simulations were done using three models. Blue lines are simulations using a model with no C pool [**see Supporting Information—Eq. S1**]. Green line are simulations using a model with only one common non-structural carbon pool [**see Supporting Information—Eqs. S9 and S10**] and pink line is the model with separate soluble sugar and starch pool [**see Supporting Information—Eqs. S15–S17**]. The environmental conditions used were: first 10 days 22 °C and 300 μmol m⁻² s⁻¹, from Day 10 to 20 average daily temperature of 15 °C and light intensity of 400 μmol m⁻² s⁻¹ and from Day 20 to 30 average daily temperature of 28 °C and light intensity of 200 μmol m⁻² s⁻¹. The photoperiod was 16 h..

20 to 30). In the end, the accumulation of total dry weight (Fig. 1D) (the sum of non-structural carbon and structural mass) is the same for the three models because C input from photosynthesis and loss by respiration is the same for all models.

In the present example, we illustrate that the inclusion of a dynamic C pool in growth models offers a more realistic representation of C allocation in abiotic stress conditions (e.g. a higher allocation to storage during sink-limited conditions), and a more realistic effect on the growth patterns. This, in principle, can allow us to test hypotheses and different combinations of environmental conditions. The third model, which is built up on concepts discussed in this paper, represents in a very basic way the result of complex enzymatic interplay. This model approach provides a basis for further development and offers possibilities for targeted experimental studies on the rates of accumulation and depletion of C and their dependence on temperature. Subsequently, model-based studies may help us to understand the effect of temporal surplus of C on the specific growth rate of organs, which is a difficult task. We believe that this simple example highlights the potential effects of including these biochemical fine-tuning mechanisms on plants.

4. OUTLOOK

Here we identify four major knowledge gaps and propose concrete steps that can advance understanding of the regulation of C storage in plants. Filling these gaps would allow modelling these processes more accurately and to quantify growth of plants in environments with fluctuating light and temperature.

First, crop growth models should explicitly include a physiology-based dynamic C storage pool (Fig. 2). This knowledge gap can be addressed by coupling biochemical models describing soluble sugars and starch metabolism (e.g. Nagele *et al.* 2010; Scialdone *et al.* 2013; Millar *et al.* 2014; Seki *et al.* 2017) to plant growth models (e.g. Rasse and Tocquin 2006; Flis *et al.* 2015). Primary carbon metabolism is highly influenced by temperature (Pyl *et al.* 2012; Pilkington *et al.* 2015), light and clock signalling (Scialdone *et al.* 2013) and these environmental factors

affect C accumulation and remobilization differently. Including separated starch and sucrose pools can increase the resolution and robustness of crop growth models, specifically if the aim is to predict growth responses in short timescales (within a day) or diurnally. This would allow testing specific hypotheses over a wider range of fluctuations; for example, the effect of alternating day lengths, extreme temperatures, or different day–night temperatures on growth and the C balance of the plant.

Second, detailed analysis of the relationship between C storage and growth across different species is needed. So far, diel starch turnover and its relation with biomass has almost exclusively been investigated in *Arabidopsis* (Stitt and Zeeman 2012; Dietze *et al.* 2014). However, it is relevant to question if the information gained from this model species is fully applicable to crops (Wiese-Klinkenberg *et al.* 2010). For example, starchless *Lotus japonicus* did grow well in a 12-h photoperiod (Welham *et al.* 2010), whereas starchless *Arabidopsis* showed a reduced growth (Rasse and Tocquin 2006). Furthermore, Wiese-Klinkenberg

et al. (2010) showed differences in diel (24 h) leaf growth between dicot and monocot plants, which points to differences in resource allocation strategies. In addition, the competition for C between an 'active' storage and other sinks changes strongly according to the phenology of the plant (T. Li *et al.* 2015). Fruits are strong sinks that compete with C storage; therefore, a relevant question would be if the fruit 'active' competition for C affects the fine-tuning of the diel sucrose–starch dynamics. These studies are important, especially if the goal would be to optimize resource use in crop production in an agricultural context.

Third, how is the information gained from diel starch turnover useful to predict C storage and acclimation of growth over a long term? Biochemical models describing starch–sucrose dynamics address acclimation of sucrose–starch partitioning and degradation to a change in photoperiods and changing temperature (e.g. Seki *et al.* 2017). However, the consequences at a whole-plant level over longer term environmental fluctuations (e.g. over weeks or months) are still missing. To address this, better
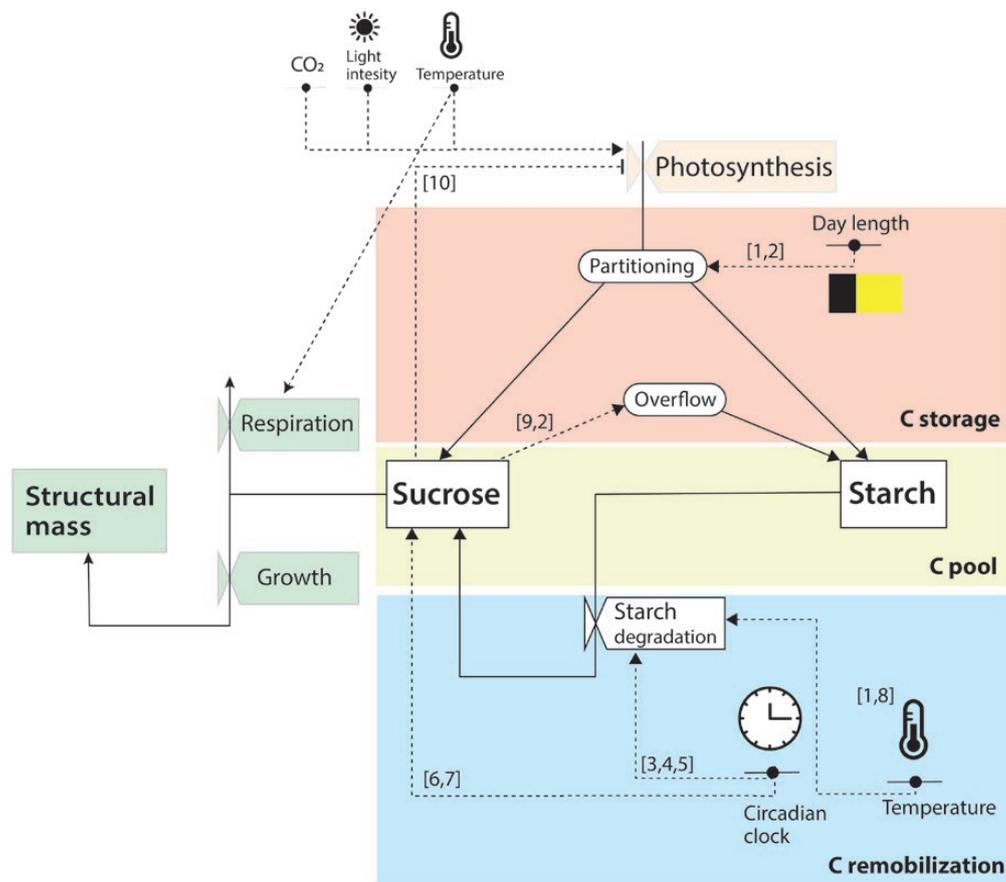

Figure 2. Schematic diagram of the model of how the C pool is influenced by day length, circadian clock and temperature. Partitioning to sucrose or starch is expressed as the proportion of fixed C that is accumulated during the light period. Starch degradation to sucrose at night is dependent on the circadian clock and is paced so that almost all starch is exhausted by dawn in source-limited conditions. Starch degradation at night can be influenced by temperature. Sucrose levels in both conditions (either source-limited or sink-limited) feedback to circadian clock to dynamically regulate the phase of the circadian oscillator. In sink-limited conditions there is less partitioning to starch. Feedback regulation decreases sucrose synthesis and triggers starch synthesis when sucrose accumulates on the leaf (overflow). Feedback downregulation of photosynthesis occurs when there is an excess accumulation of sucrose in the leaf. In sink-limited conditions not all the starch is completely remobilized at dawn and high temperature can accelerate the rate of starch degradation. Rectangles indicate states, valves indicate rates, solid lines indicate mass flows and dotted lines indicate flow of information. Numbers indicate references in support of the model as follows: [1] (Pilkington *et al.* 2015); [2] (Mengin *et al.* 2017); [3] (Gibon *et al.* 2004); [4] (Scialdone *et al.* 2013); [5] (Graf *et al.* 2010a, b); [6] (Feugier *et al.* 2013); [7] (Seki *et al.* 2017); [8] (Müller *et al.* 2018); [9] (Horton 1985); [10] (Paul and Foyer 2001).

reference data for model evaluation at a whole-plant level are needed, and these data set should include C assimilation (gas exchange measurements), quantification of primary metabolites and growth rates. Additionally, conducting experiments with long-term temperature and light fluctuations would provide valuable information on longer term mechanisms of C storage and the consequences on growth.

Finally, if the goal is to increase productivity of crop plants while making a more efficient use of resources (e.g. water, nutrients, supplemental light, CO_2), a multiscale approach linking C metabolism at cell level with whole-plant growth is needed. By using isotope labelling techniques, metabolic network fluxes can be estimated and incorporated into a model that would allow simulation of the effects of a dynamic environment on C metabolism in plants and plant growth (Sweetlove *et al.* 2017) or to determine differences between 'readily available' and long-term pools. A fundamental challenge in multiscale prediction is that the model becomes more complex when moving across molecular or biochemical scales to whole organisms due to the different timescale and finer granularity of the processes (Hammer *et al.* 2004). This leads to difficulties related to parameter identification or computational time for optimization (Hammer *et al.* 2019). To successfully bridge across levels, the biochemical process (in this case the starch–sucrose dynamics) must have robust and quantitative measures of physiological responses. A balance between process details and predictability of the system in scales that are feasible to measure must also be achieved (Peng *et al.* 2020). A multiscale model that operates effectively across the biochemistry level and whole-plant level would provide an important tool to rapidly assess different manipulation in the C partitioning or C storage and to explore different strategies for the control of the environmental conditions in which plants are growing.

5. CONCLUSION

The efficient management of C storage is essential for plant growth to be optimized, especially under fluctuating environmental conditions. As conducting experiments with many different light levels and temperatures is often not feasible, crop modelling can be used as a tool for hypothesis testing and scenario analysis under varied environmental conditions. To achieve this, crop growth models must extend beyond current scales and include a finer detail in essential processes like C storage and C usage and remobilization. Including separated starch and sucrose pools to represent an 'active' pool in growth models can increase the level of detail and robustness of the models. This is essential for predicting growth response on short timescale in ever-fluctuating environments. Scenario-based model simulations can potentially guide manipulation and improvement of C allocation strategies (trade-off between C storage and other sinks) and can help to explore opportunities to optimize crop performance under temporal unfavourable environmental conditions and thus for developing more resource-efficient crop production systems.

SUPPORTING INFORMATION

The following additional information is available in the online version of this article—

CONFLICT OF INTEREST

None declared.

CONTRIBUTIONS BY THE AUTHORS

Conceptualization A.C.Z., E.H. and L.F.M.M., A.C.Z. wrote this manuscript and E.H., L.F.M.M. made substantial contributions to improve the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The data that support this study will be shared upon request to the corresponding author.

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