

Deciding When and Where to Grow

Perspectives on C/N Balance

This is a writing assignment for the Masters course Bioinformatics and Biocomplexity.

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Abstract

This literature review discusses the intricate relationship between carbon (C) and nitrogen (N) in vascular plants. In order to grow and reproduce optimally, plants must maintain an internal balance between C and N while responding to changes in external availability. Although the C and N metabolic pathways are individually well-studied, mechanisms for regulating C and N levels are complex and multi-levelled, and several important details regarding how the C/N pathways interact to maintain balance are still unclear. This review examines how different fields in biology view the problem of resource allocation such as evolutionary biology, developmental biology and biophysics, using representative examples to show the strengths and weaknesses of each perspective. Furthermore, we highlight some key questions to be answered by each field to form a unifying picture of the system as a whole. The review concludes by suggesting that a combined approach is needed to inform future models, and gives some suggestions on what aspects of current perspectives present useful properties to be considered for such models. Special emphasis is given to the interpretation of signals, and a need for deeper inquiry into how a signalling molecule's spatiotemporal dynamics and functional involvement in the C/N metabolic pathway can affect the potential information it carries to inform regulation processes.

Lay Summary

Carbon (C) and nitrogen (N) are essential to all life on Earth. While animals must consume organic material in order to obtain C and N, plants can absorb inorganic C and N in their surroundings. This makes plants the primary producers of food for many ecosystems, so understanding how plants absorb and regulate internal amounts of C and N has significant implications for biology. However, while the processes of how plants absorb external C and N are well-studied, the mechanisms they use to regulate levels of C and N internally are less well understood.

Researchers from diverse fields have investigated the mechanisms that plants use to control internal C/N from different perspectives. This approach has helped to gain a more focused understanding of the various components necessary for regulating C/N balance, such as signalling network structure, physiology, and growth strategies. However, focusing solely on specific aspects of how plants allocate their resources is insufficient to fully understand the complex system. Plants use multiple interacting components that work on different scales of time and space to allocate resources. Thus, to gain a more complete understanding of resource allocation in plants, we must consider all of these different components and how they interact.

To illustrate this point, we use representative examples from three fields of biology: evolutionary, developmental, and physical. Each gives a unique perspective on how plants regulate their internal C and N levels: evolutionary biology focuses on optimal strategies plants use to maximise resource efficiency; developmental biology considers how plants produce signalling compounds that influence growth and development; biophysics looks at how physical properties such as shape and resource concentrations influence resource uptake and transport. We show that despite these different perspectives, there are still gaps in consensus knowledge. For example, we highlight that descriptions of signalling networks neglect the physical properties of signalling molecules, as well as how these molecules may be involved in the processes of resource acquisition.

An integrative framework is needed to adequately describe the mechanisms behind C/N balance in plants. However, this is difficult because of the gaps in consensus knowledge, especially regarding how signalling molecules allow plants to communicate information. Further research is needed to fill these gaps and fully understand the complex mechanisms that govern C/N balance in plants.

In summary, this literature review provides a consensus understanding of how vascular plants balance internal C and N levels, highlighting key unanswered questions and the need for an integrative framework that takes a multidisciplinary approach to combine the strengths of current knowledge. Further research is needed to fully understand the complex mechanisms that govern C/N balance in plants, which could have significant implications for agriculture and environmental management.

Introduction

Carbon (C) and nitrogen (N) are essential elements that form the foundation for all cellular components, hence the processes through which C and N are integrated into biological systems significantly influence all known life. As autotrophs, plants are able to absorb inorganic C and N and therefore serve a crucial role in the assimilation and cycling of C and N within ecosystems. Gaining a deeper understanding of how plants control C and N uptake and allocation thus has huge ecological implications, potentially shedding light on the intricate relationships within ecosystems and informing our management of natural resources.

Vascular plants absorb C and N through the leaves and roots, respectively, organs on opposite extremes of the body exposed to different local conditions. Despite the distance between them, the leaves and roots are interconnected and the processes of gathering available C and N from the environment are intrinsically dependent on each other. C is mainly absorbed as inorganic carbon dioxide and assimilated via photosynthesis through N-based proteins; indeed the majority of N found in the leaves is involved with photosynthesis in the form of Rubisco (Evans and Clarke 2019). In turn, N is absorbed via the roots, which use C-based sugars and polysaccharides as energy and building materials, as well as providing C storage (Kell 2012). Maintaining an appropriate C/N balance is crucial for a plant's survival and productivity, an imbalance can lead to reduced photosynthesis, poor growth and increased susceptibility to stress; thus to optimise fitness and adapt to environmental changes, plants employ various strategies to maintain the proper C/N balance through growth, physiology and adaptation mechanisms.

In order to maintain this balance, plants are capable of coordinated responses to C/N availability. For example, optimal resource availability at one end of the plant usually stimulates growth in the other end (Hachiya et al. 2014; Luo, Zhang, and Xu 2020), indicating systemic mechanisms to communicate resource availability. Moreover, organs can grow preferentially in regions where resources are abundant: root growth is prioritised in branches where nitrate is discovered (Giehl and von Wirén 2014), while local light signalling in leaves induces differential petiole growth (Küpers et al. 2023). However, the ability to focus resources locally is altered by systemic signals depending on how nutrients are distributed in other parts of the plant. The intricate signalling networks that control physiological and growth responses to C/N status have been extensively researched through the likes of genetic studies and biochemical assays, and many physiological processes and molecular players have been identified (Baslam et al. 2020). Despite this progress, the underlying logic behind how growth and nutrient allocation are controlled in plants is not yet fully understood. Further research is necessary to fill these knowledge gaps and develop a comprehensive understanding of this complex system.

Since resource allocation is a multilevel process, different fields of biology tend to view the problem from separate angles: eco-evolutionary models consider whole-plant optimisation; developmental biology focuses on how signalling

molecules inform cellular growth and development; while biophysicists may look at how resource properties, concentration gradients and internal geometry affect vascular transport between organs. This literature review aims to give an overview of these different viewpoints while indicating their strengths and weaknesses. It makes reference to representative examples that look at where information is available and integrated, how the nature of signals affects the type of information they carry and the constraints imposed by the biophysics and architecture of plants. We end with a summary of how perspectives' strengths could be combined to inform future models, viewing C/N balance as a distributed information processing problem that operates within the embodiment of a plant in its environment in which resource acquisition and transport is governed by the biophysics of the vascular system.

Different Perspectives on C/N Balance

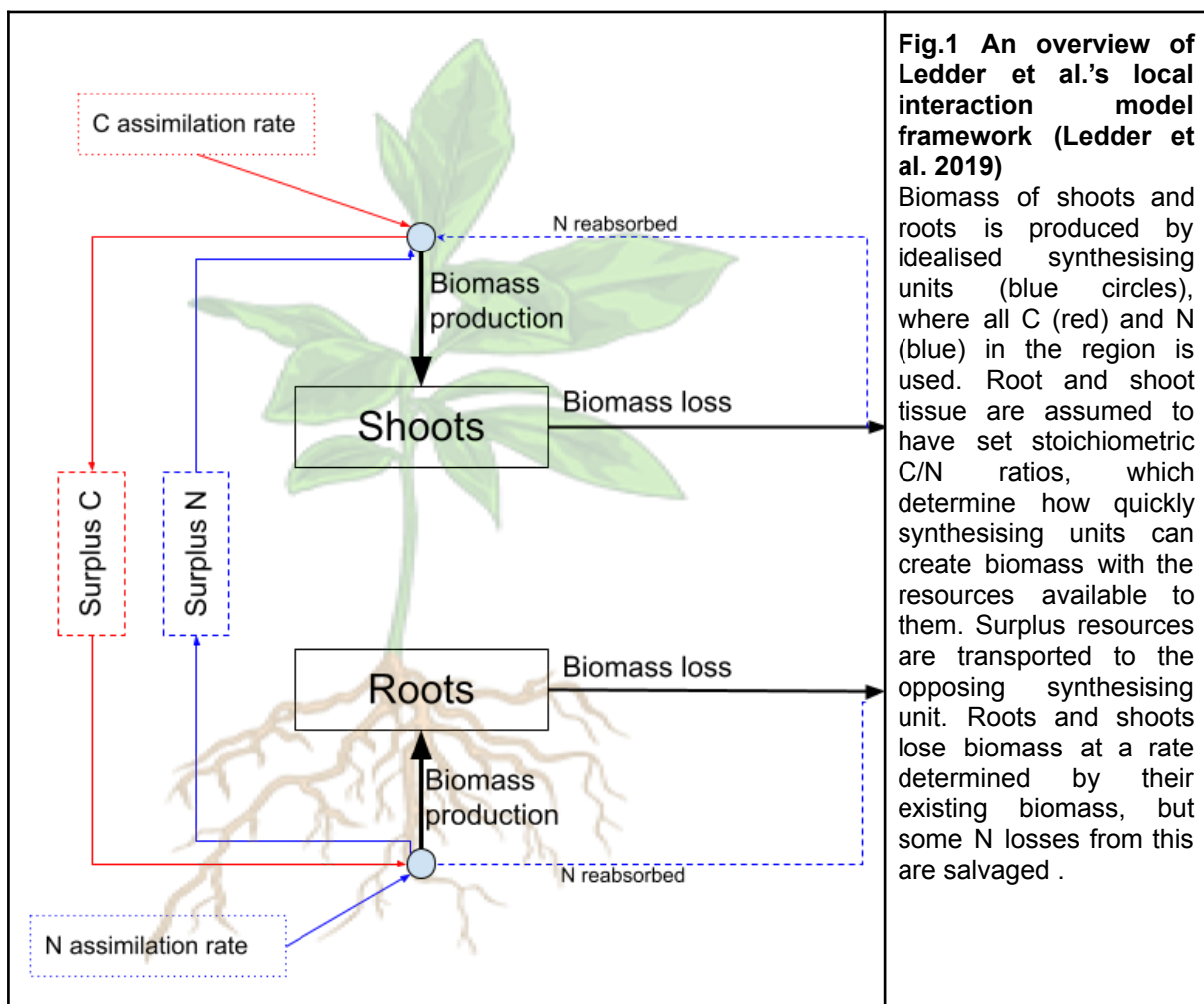
Optimisation - Are whole-plant strategies possible?

Evolutionary frameworks of resource allocation observe that strategies have evolved in response to the selective pressure of outcompeting other plants, leading to the common assumption that plants behave optimally in their environment. For example, an influential evolutionary approach is the balanced growth hypothesis, an idea inspired by economic theories on optimal resource allocation that suggests plants adjust growth rates and nutrient acquisition strategies to maintain a relatively constant ratio of resources in their tissues in order to achieve optimal growth (Lambers 1983; Bloom, Chapin, and Mooney 1985), although mechanistically how this is implemented is unclear. While there can be a lot of nuance in what 'optimal growth' actually entails, many models use whole-plant growth or reproductive rate as a proxy (Bloom, Chapin, and Mooney 1985; Franklin et al. 2012; Wilson 1988), calculating how these are maximised by allocating resources to local growth processes from an assumed whole-plant C/N resource pool. Under optimal growth conditions, the hypothesis states that resources are allocated to growth processes to become equally limiting, meaning no single resource is in excess or is a bottleneck for the plant's growth (Bloom, Chapin, and Mooney 1985); a behaviour which has been observed experimentally (Poorter and Nagel 2000). Alternatives to the balanced growth hypothesis such as resource competition (Tilman 1982) or dynamic allocation (Müller, Schmid, and Weiner 2000) argue that environmental heterogeneity such as variable resource availability or herbivore presence lead to deviations from balanced growth by forcing trade-offs between root/shoot growth when competing for foraged nutrients and/or requiring investment into defence (Monson et al. 2022), but these hypotheses still share the same assumption present in the balanced growth hypothesis that plants are able to exert control over a collective resource pool on the scale of the entire organism, in order to achieve an optimal allocation solution.

The assumption that plants can exert global control over resource allocation is problematic: this would imply that plants are able to assess the whole-plant resource and demand status, but the mechanisms which plants would use to do so have not

yet been described. Furthermore, plants lack a central organ to hold and integrate global information, like a brain, and there is currently no description of what would function as a global controller in its place. Despite lacking a brain, plants show behaviour that superficially resembles capabilities for memory (Gagliano et al. 2014) and even prediction (Calvo and Friston 2017), which indicate the capability to perform some form of information-based processing. This lack of understanding makes it uncertain how plants can effectively coordinate and allocate resources across different organs and physiological processes to achieve optimal growth and reproduction.

Taking an alternative perspective on how optimality can be obtained, Ledder et al. built a framework to investigate whether an optimal solution for C/N allocation can emerge from strictly local rules without the need for global control (Ledder et al. 2019). They argue since roots and shoots show some independent patterns of growth but gather complementary shared resources, their relationship can be likened to the syntrophic (metabolically joined) symbiosis between individuals in a holobiont. Inspired by models of reef coral and intracellular photosynthesising dinoflagellates (Cunning et al. 2017), a framework is built on the key assumption that roots and shoots operate selfishly to maximise personal growth needs with the resources 'locally' available to them before transporting surplus to their partner (Fig.1).



Ledder et al. make an important point about needing to understand how plants achieve optimal allocation in the absence of global control, but the claim that their framework demonstrates a solution without global control is debatable. While not immediately apparent, the model implicitly contains global control. For example, it sets overall shoot and root tissue growth to require fixed C/N stoichiometries, but in practice internal stoichiometries can vary depending on environmental conditions (Sternler and Elser 2002) so imposing this fixed ratio is a form of global control. In addition, since the two idealised growth processes represent the growth of branching organs, assuming the plant is able to assess what resources are surplus to send to the opposite end technically requires some form of interpretation, giving global information on C/N status and thus can be considered a form of global control. Since the framework solves an ordinary differential equation for the whole plant, growth rates are still tuned to global availability demands for an optimal solution, something the framework is attempting to avoid.

In addition to not solving the issue of global control, their model has noticeable biological shortcomings. It attempts to scale up a previous model describing metabolic interactions in a small holobiont with two interacting organisms, where it was relevant to describe the growth of each organism originating from a single point; but to do so for roots and shoots is unrealistic (perhaps besides very early developmental phases). Leaves and roots grow through a branching process that spatially distributes the organs in the local environment for foraging purposes (as well as balance and stability) (Fitter and Hay 2002) and growth rate can be highly dependent on internal geometry in addition to tissue type (Kierzkowski and Routier-Kierzkowska 2019). Furthermore, the model's claim to focus on local dynamics of resource acquisition and utilisation is appealing, but the framework only considers 'locality' to the extent of splitting into roots and shoots, whereas acquisition processes can occur with more specific locality. For example, N is absorbed in the form of nitrates but also ammonium, which typically occupy different soil levels (Schuster et al. 2023); plants develop primary and adventitious root systems at different soil heights to access different nutrients which do not directly transport between each other.

Despite these critiques, the framework still provides a valuable perspective on C/N balance by confronting a common, but non-robust assumption taken by many evolutionary perspectives that plants have global knowledge of their internal status. The proposition that plants may not need global knowledge to achieve an optimum is an interesting potential solution, though unfortunately Ledder et al.'s model does not demonstrate how this could be done realistically. Nevertheless it still indicates a need to assess how plants can actually achieve a global optimum, and leads one to speculate to what extent the dynamics of allocation behaviour are determined by optimality in practice.

Mutual Signalling - What does a signal mean?

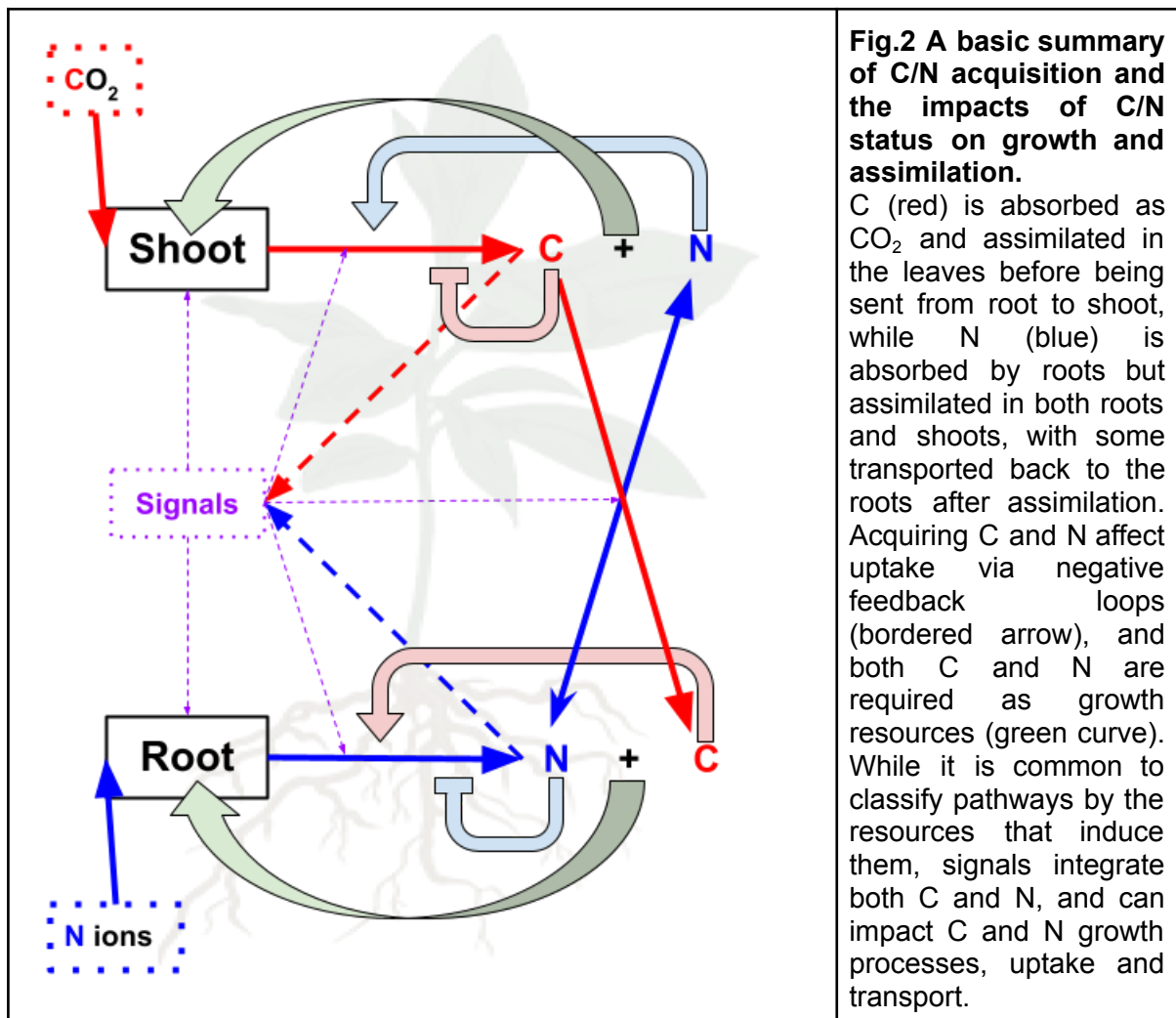
Developmental approaches to studying plants typically focus on the detailed molecular, cellular and tissue-level processes that govern growth and development, providing a more fine-grained perspective compared to evolutionary approaches. When examining resource allocation through a developmental lens, emphasis is placed on the signalling networks that play a crucial role in managing growth and development. In contrast to evolutionary approaches, examining allocation from the perspective of signalling networks does not require the assumption of optimal plant performance. Instead, it adopts a more biomolecular approach, where researchers observe correlations between the presence of suspected signalling molecules and growth processes to infer the signalling cues they provide to cells.

As the fundamental units of a living organism, cells are equipped with a multitude of receptors and molecular machines that enable them to receive, process and respond to various signals and environmental cues (Alberts et al. 2015). In this context, cells function as basic information processors: they take inputs in the form of molecular signals, process this signal as information, and produce a specific output or response constrained by their current resource levels. Signalling molecules impact the production and transport of other signals to form networks that can act over both short and long distances (Brackmann and Greb 2014). As such, signalling networks serve as a means for plants to distribute information to coordinate and fine-tune various cellular and developmental processes, including C/N balance (Coruzzi and Bush 2001).

Split into resource and signalling properties, the system of C and N allocation is composed of three main components (Fig.2). First, both C and N are required as resources for all growth processes, but the impact of growth on acquisition capability is organ-specific: growth in the shoots enhances CO₂ uptake, while growth in the roots increases the potential amount of N ions absorbed (Poorter et al. 2012). Second, C and N serve as resources for uptake and assimilation; each inhibits their own local uptake through saturation or toxic accumulation (Paul and Foyer 2001; Britto and Kronzucker 2002; Dechorgnat et al. 2011) and stimulates uptake of the other; such as C providing available energy for N transport and N enhancing efficiency of photosynthesis (Bloom, Chapin, and Mooney 1985). Finally, in addition to the resource needs of C and N for growth, their intake also stimulates highly complex, dynamic signalling networks that communicate between shoot and root (Vercruyssen et al. 2011). Coordination of growth between shoot and root is necessary for a plant's proper development for reasons such as structural stability (Telewski 2006), but since growth can have different impacts on C and N depending on location, modulating where and when it happens can also control C/N balance. In addition to controlling C/N balance through growth responses, signals can also influence rates of uptake, transport and assimilation.

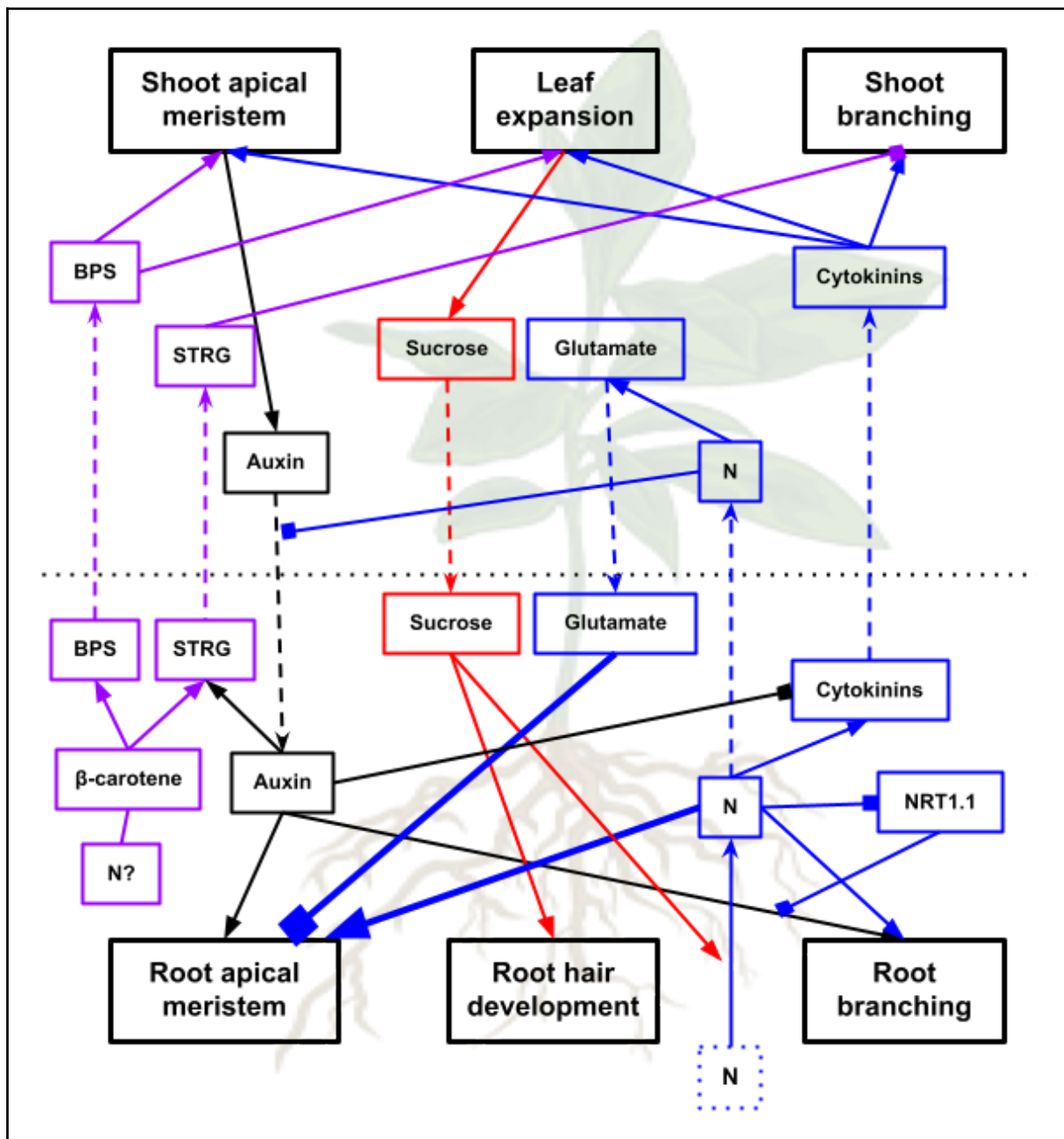
Although signals are often classified based on the resources that induce their production (with the intent of trying to label what information their presence signals), many signals in the C and N network are affected by both metabolic pathways (Puig

et al. 2012). Furthermore, signalling molecules can also affect both C and N processes, for example trehalose-6-phosphate (Tre6P) is a sugar-phosphate molecule thought to signal sucrose availability that influences internal C in several ways, such as inhibiting the breakdown of starch into sucrose (Lunn et al. 2014); but it is also involved in the regulation of N uptake and assimilation (Figuroa et al. 2016). Given that signalling molecules can travel long distances through the plant (Brackmann and Greb 2014), the networks they form provide a potential means for how plants process local information given by resource acquisition and assimilation in order to inform processes that globally balance C and N, but the complexities of how signalling networks allow plants to process information are still poorly understood (Gagliano et al. 2014). Nevertheless, it is clear that in conjunction with the interconnected needs for C and N and their influence on growth, uptake and transport, signalling networks are critical to a plant's ability to respond to environmental changes without a brain (Calvo and Friston 2017).



The abundance of molecular data currently available allows researchers to integrate and analyse signalling networks, incorporating newly identified pathways to continuously update the pre-existing consensus framework. For example, Puig et al.

developed a framework (Fig.3) by integrating identified pathways involving carotenoid-derived hormones with known signalling processes of auxin, cytokinin and sugar¹ (Puig et al. 2012). Their study documents experimentally observed growth responses resulting from these signalling networks, as well as knowledge of how the networks affect each other through signalling molecules that up/downregulate the transport and production of others. To distinguish between the differences in observed growth responses, growth in the roots and shoots was divided into three processes: apical meristem growth, development of absorbent surface area (leaf expansion/root hair development) and branching.



¹ They also consider a pathway stimulated by absorbing P, but this has been ignored for this review to focus on C/N balance.

Fig.3 Overview schematic of Puig et al.'s conceptual framework (P removed) (Puig et al. 2012)

Pointed arrows indicate stimulation, while square arrows indicate inhibition. Signals that travel from shoot to root (or vice versa) indicated by dashed lines. Colour indicates the main origin of signals, with C in red, N in blue, auxin (which interacts with N) in black and carotenoids in purple. *BYPASS1* (BPS) and Strigolactones (STRG) are part of a more recently discovered pathway, which has here been added to more well-studied network interactions, but key details such as what starts beta-carotene signalling are still not known. N and glutamate signalling effects on root apical meristem growth are emboldened to highlight that different signals within the same resource signalling network can have opposing effects.

Puig et al. do not aim to provide an entire picture of the consensus view of C/N signalling, instead giving an example of how new discoveries can be integrated into previously known networks. As such, the framework only minimally considers C signalling (in the form of sucrose) and does not discuss some important details such as the CEP pathway, which is induced by N in the shoot and transmits a signal from shoot to root to regulate N uptake (Chapman et al. 2020; Ota et al. 2020). However, the pathways arranged into a signalling network graph as shown in Fig.3 still give useful demonstrations of representative systemic behaviour seen in C/N signalling networks mentioned earlier and outlined in Fig.2.

First, we see that several signalling molecules travel between the two organs (shown by dashed arrows), this can be from shoot to root (auxin, sucrose and glutamate) or root to shoot (*BYPASS1*, strigolactone, nitrate and cytokinins). All the long-distance signals shown have some influence on observed growth patterns at the end they travel to, but each influences a different set of the three growth types, showing that growth responses to signals are complex and location-dependent; while N impact on growth in the shoot is not shown in this framework, it induces further signalling networks in the shoot that aren't shown in Puig et al.'s framework (Wang, Hsu, and Tsay 2012). Next, it shows that while a network is formed of signals that up/downregulate the production of others and influence growth processes, the signals can also influence each other by changing transport (such as N inhibiting auxin transport), or even uptake, if the signal is an absorbed resource (such as sucrose increasing N uptake). It shows that signals given downstream in the same resource network can have opposing effects to provide negative feedback: N in the root increases root apical meristem growth, but also travels to the shoot to aid in the synthesis of glutamate, which travels back to the root and decreases root apical meristem growth.

Fig.3 also shows that there are still clear gaps in new networks; for example, what induces the production of beta-carotene in the root is unclear, though previous studies have linked beta-carotene to N availability, but in the leaves (Yan et al. 2015). Furthermore, Puig et al. admit that consensus knowledge of the better studied networks are still "fragmented", but some key details such as glutamate's role in linking C and N metabolisms for plants are now better described (Qiu et al. 2020). However, aside from the need to update networks with new information of putative

signalling pathways, the network diagram in Fig.3 does not make two aspects of signalling clear: labelling a molecule as a signal does not consider the context of their biological function, and it is non-trivial consider how the transport of signals and growth processes are linked (this latter point will be addressed during the next section).

Of the 7 long-distance signals described, 4 (BPS, STRG, auxin and cytokinin) are hormones explicitly produced for signalling, sucrose is a sugar produced as an energy resource, glutamate is an amino acid and nitrate is an inorganic absorbed ion. Yet, an approach that categorises molecules through measuring their correlation to growth processes would call all of them 'signals', to indicate their presence provides information to regulate a growth process. Returning to the idea of cells as information processors, calling a molecule a 'signal' implies that it gives information to inform a cellular process (Scheres and van der Putten 2017). However, differences in properties of signalling molecules may influence the information that their presence can provide.

For example, glucose availability is signalled by chemical signals like hexokinase (de Jong et al. 2014), but glucose availability can also be indicated by high local concentrations of glucose itself improving source strength (White et al. 2016). There is a fundamental difference between these two C signals : glucose concentration is dependent on production, consumption and retrieval from sucrose storage, processes that its presence as a signal influences; whereas hexokinase is an enzyme that signals C availability indirectly by modulating its enzymatic activity in the presence of high glucose levels, thus is not directly consumed (though it does still decay). In either case, their function affects the time scales at which they operate, which will affect the information they can carry. For example, signals that are short-lived may provide more information of current status, or be more suitable for informing localised growth; this is important to consider given that processes such as lateral plant growth require both long and short-distance signals (Brackmann and Greb 2014).

A signal's different functions and temporal dynamics dictate the information they can potentially provide. To demonstrate this, let us examine a basic overview of C assimilation (Fig. 4), and consider three key molecules involved in the C signalling pathway: sucrose, Tre6P and ELONGATED HYPOCOTYL5 (Hy5). Sucrose is an important energy resource produced by photosynthesis to be taken to sink tissues for energy use and storage; it additionally functions as a C signal to upregulate various growth processes (Stein and Granot 2019). Tre6P is a phosphorylated sugar that signals C availability by inhibiting SnRK1 to promote growth (Gazzarrini and Tsai 2014) as well as regulating sucrose levels through a negative feedback response (Figuroa and Lunn 2016). Finally, Hy5 is a transcription factor that signals the presence of light, indirectly regulating C levels through influencing the expression of genes related to photosynthesis to promote C assimilation, but more recently it has also been found to stimulate root growth (Chen et al. 2016); since N is assimilated in many plants using a light-assisted process, this provides a potential explanation for why Hy5 might be involved.

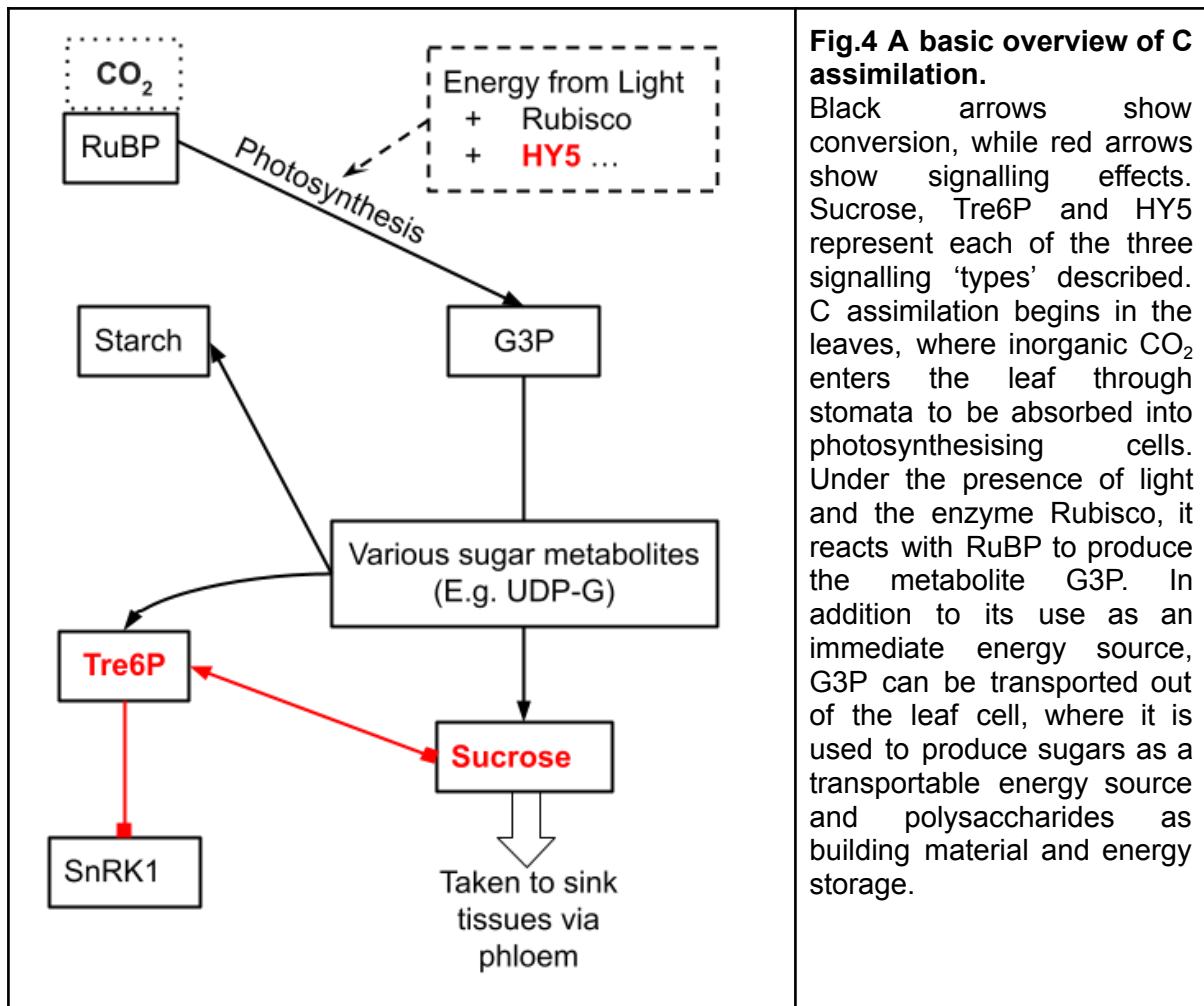


Fig.4 A basic overview of C assimilation.

Black arrows show conversion, while red arrows show signalling effects. Sucrose, Tre6P and HY5 represent each of the three signalling 'types' described. C assimilation begins in the leaves, where inorganic CO_2 enters the leaf through stomata to be absorbed into photosynthesising cells. Under the presence of light and the enzyme Rubisco, it reacts with RuBP to produce the metabolite G3P. In addition to its use as an immediate energy source, G3P can be transported out of the leaf cell, where it is used to produce sugars as a transportable energy source and polysaccharides as building material and energy storage.

We suggest that these three examples taken from C signalling may be used to represent three simple categories of signal involvement for a general resource assimilation process, with the goal of generalising how a signal's involvement in a metabolic process may affect the information it represents:

1. 'Primary' signals are products with important metabolic uses that are stored/consumed as material resources, such as sucrose for C, or glutamate for N. Since sucrose is consumed by growth processes or sent to energy storage (either as a sugar or by conversion to starch), the amount of sucrose locally present may give a signal for what is immediately possible, but present little information on the current rate of photosynthesis or overall C status. Similarly, glutamate is a signal that serves as a key nitrogen donor in a variety of metabolic pathways (Forde and Lea 2007; Qiu et al. 2020), but is also stored long-term either as-is or through conversion to other stable amino acids, impacting what its presence as a signal for N status represents.
2. 'Secondary' signals are linked to the production of a primary metabolite, but not directly consumed by the responses they signal for, such as Tre6P for C, or nitric oxide (NO) for N. Tre6P and sucrose production are linked through

the shared use of UDP-G as a precursor, but Tre6P does not function as an energy source, instead mainly functioning as a signal². While Tre6P signals sucrose availability, plants show different responses to its presence than to sucrose itself: for example, plants retrieve sucrose from starch storage overnight to supply energy while they aren't photosynthesising, and Tre6P inhibits this retrieval; experiments that artificially increased Tre6P synthesis during the day observed significantly reduced starch breakdown at night despite low sucrose levels (Martins et al. 2013), indicating that Tre6P provides information on daily photosynthesis success to indicate sucrose excess. Since secondary signals are linked to primary signals through a production process, this implies the possibility for expected ratios of production between primary and secondary resources (as can be seen for Tre6P and sucrose. If this ratio increased under resource limiting conditions, then plants could potentially use secondary signals to provide information on resource limitation (which primary signals cannot). For example, while the production and signalling of NO in plants is still poorly understood, its production during nitrate assimilation seems to be upregulated during N limiting conditions and it can signal physiological and metabolic responses that reduce the plant's N demands (Baudouin and Hancock 2014).

3. Sensory signals that are produced to act upon a metabolic process (i.e. transcription factors and enzymes), such as hexokinase (for C) or Hy5 (for both C/N). These are not explicitly produced by the metabolic pathway they influence (although of course since we are discussing C/N balance, virtually everything is implicitly produced by it downstream) For example, Hy5 is a transcription factor that regulates gene expression in response to light, and is implied as a light-sensor. Viewing it as such gives context to the responses it induces: why it might stimulate photosynthesis is obvious, but it has also been shown to provide signals for root growth (Chen et al. 2016). In many species, a significant amount of nitrate is assimilated in the leaves through a light-assisted process, we can speculate that such functional links are what allow networks based on sensory signals to form.

This is perhaps an overly broad simplification, since as we have shown, signalling molecules can be involved in multiple pathways to mediate different responses as well as integrate information on multiple resources. In addition, since this work focuses on C/N balance, this analogy is to help categorise signals for presence/availability, and some responses to other processes may be difficult to adjust to this context: for example, UDP-G is involved in the C metabolism but also acts as a signal for cell damage (Janse van Rensburg and Van den Ende 2018). Nevertheless, we hope to have sufficiently demonstrated how frameworks that solely consider a network graph with connections to describe resource signals are

² Tre6P also influences many other pathways such as preventing desiccation during times of drought, but these are not yet fully understood.

insufficient to understand what information these signals represent and how plants respond to them.

Vascular Dynamics - How do biophysical properties influence resource allocation?

The two perspectives shown thus far have explored resource allocation strategies, representing a whole-plant level process, and how the signalling networks involved in mediating these strategies are structured, which describe how cell-level processes are informed by stimulus received from far away. However, combining these two perspectives requires understanding how signals are directed by transport at the organ-level, that is to say the influence of biophysical properties such as mechanics of vascular transport and its impact on the spatial dynamics of signals. These practical details cannot be trivially ignored; they are not merely the mechanical implementation of growth signalling/responses, but instead directly influence the information processes of resource allocation in their own right. Contemporary models of resource allocation which explicitly incorporate spatial structure and biophysics in addition to signalling pathways show contrasting results to earlier work that neglected such details (Herik and Tusscher 2022).

To describe the interplay between transport and signalling, White et al. proposed a framework (Fig. 5) that emphasises the effects of signals and resource concentrations on vascular transport. It describes how signals given by molecules and uptake of resources can influence local concentrations in the root and shoot, affecting concentration gradients through the vascular system that impact speed of transport via source-sink dynamics (White et al. 2016); Shoots are a C source and an N sink, while roots are the opposite. However, White et al.'s framework still leaves some room to be expanded upon, while effects of resource uptake on concentration gradients are convincing, their description of signals and how they affect the sources and sinks is still somewhat limited; signals are considered as only giving information on C or N exclusively, which disregards the close relationship between C and N signals described previous sections. One example describing a physical process that links C and N processes for a signalling molecule is nitrate; nitrate uptake in the roots increases source strength, but nitrate in the leaves has been shown to induce stomatal opening (Guo, Young, and Crawford 2003) which increases transpiration, increasing the rate of flow through the xylem; however, opening the stomata also improves the rate of CO₂ absorption, increasing C source strength and thereby linking the physical processes of N and C absorption.

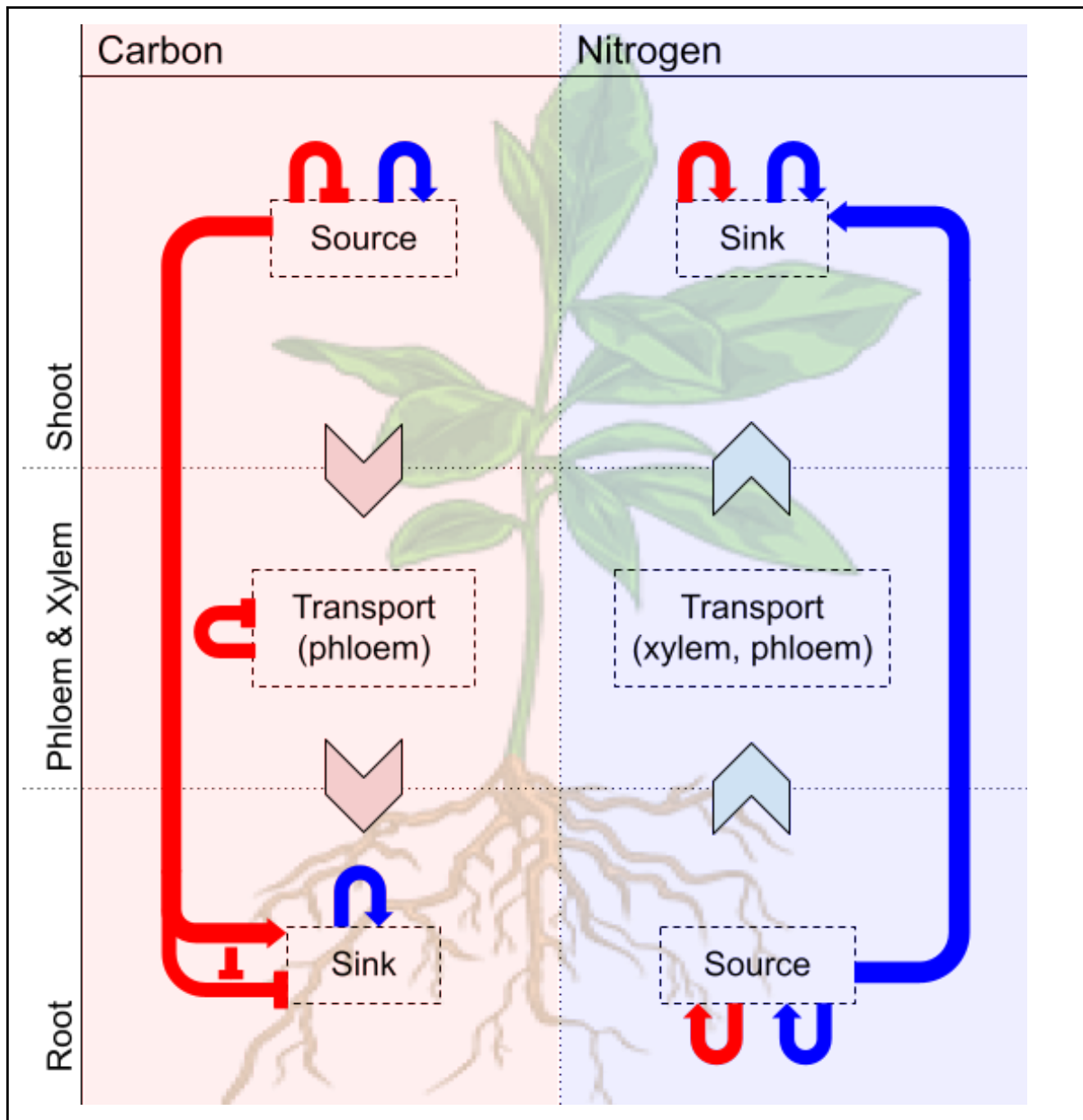


Fig.5 An overview of White et al.'s source/sink framework (White et al. 2016)

It shows the shoot as a C source/N sink and roots as an N source/C sink. Coloured arrows between organs represent signalling that regulates source/sink strength, with C in red and N in blue.

In addition, while this framework focuses on source/sink dynamics for C and N, it has limited consideration of how vascular properties affect the transport of non-primary signalling molecules, which would help to understand how signalling networks such as that of Puig et al. can send signals to specific locations to regulate local growth patterns. Modelling plants with explicit considerations of vasculature spatial structure have also shown that undirected signals can give directed effects (Herik and Tusscher 2022). White et al.'s framework still leaves questions about the impacts of spatial structure unanswered, for example, what is the effect on resource supply if two sinks are of equal strength, but different distances from a source - does

one always receive more, and if not then how do plants adjust sink strength to account for this. However, while the framework itself is overly simplistic, we strongly agree with the final conclusions of the article that a holistic perspective is required to satisfactorily describe C/N balance regulation.

Discussion

Combining Perspectives Can Improve Future Understanding

In summary, we hope to have shown that current perspectives describing resource allocation each provide their own, valuable insights into understanding how plants achieve C/N balance, through representing how C and N are regulated at different levels of organisation from whole-plant to cell level. Evolutionary perspectives help contextualise observed responses to external and internal C/N balance by interpreting what objectives those responses may have, developmental perspectives help link how stimulus received in local parts of the organ can be processed and distributed as signals through the plant to then provide information over long distances, and biophysical perspectives can assist with understanding how these distributed signals are then directed locally to induce localised growth processes to describe behaviours such as preferential foraging. As a multilevel process, understanding the overall logic of how C and N decisions are made requires an integration of these three perspectives, which though disparate at first glance, are closely linked.

We propose a system for categorising signalling molecules into one of 3 groups depending on whether they are: a primary resource; a non-primary resource produced by a process that makes a primary resource; or a sensory signal such as a transcriptional factor or an enzyme. Performing this categorisation, we hope to gain better understanding of how the function of signalling molecules can impact the information they represent for regulating processes such as growth under conditions with different resource constraints on C and N; this categorisation could also potentially help understand how signals affect biophysical properties such as vascular transport, considering how signalling molecules are linked to processes of resource consumption with more nuance.

However, as we have seen in this review, there are still gaps in consensus knowledge that make an integrative framework difficult to pin down. We suggest it especially important for future experimental research to investigate the spatiotemporal dynamics of some key signals in the C/N metabolic pathways; this combined with careful consideration of their functional involvement should improve our understanding of the information signalling molecules may represent, and see whether the system of primary/secondary/sensory can be applied to previously unseen pathways while displaying any similar properties.

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