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Tansley review

What determines transfer of carbon from plants to mycorrhizal fungi?

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Summary

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Biological Market Models are common evolutionary frameworks to understand the maintenance of mutualism in mycorrhizas. 'Surplus C' hypotheses provide an alternative framework where stoichiometry and source-sink dynamics govern mycorrhizal function. A critical difference between these frameworks is whether carbon transfer from plants is regulated by nutrient transfer from fungi or through source-sink dynamics. In this review, we: provide a historical perspective; summarize studies that asked whether plants transfer more carbon to fungi that transfer more nutrients; conduct a meta-analysis to assess whether mycorrhizal plant growth suppressions are related to carbon transfer; and review literature on cellular mechanisms for carbon transfer. In sum, current knowledge does not indicate that carbon transfer from plants is directly regulated by nutrient delivery from fungi. Further, mycorrhizal plant growth responses were linked to nutrient uptake rather than carbon transfer. These findings are more consistent with 'Surplus C' hypotheses than Biological Market Models. However, we also identify research gaps, and future research may uncover a mechanism directly linking carbon and nutrient transfer. Until then, we urge caution when applying economic terminology to describe mycorrhizas. We present a synthesis of ideas, consider knowledge gaps, and suggest experiments to advance the field.

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I. Introduction

Within mycorrhizas, carbon moves from plants to fungi and nutrients move from fungi to plants. The amount of resources transferred can be sizable, and ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) fungi are estimated to receive c. 13% and 6%, respectively, of the carbon assimilated by the plant (Hawkins et al., 2023). Similarly, AM fungi can deliver almost all the phosphorus and c. 20% of nitrogen needed by plants (Smith et al., 2003; van der Heijden & Horton, 2009) compared with 80% nitrogen and 70% of phosphorus by EcM fungi (van der Heijden & Horton, 2009). The amounts of resources transferred are not fixed but vary with environmental context (Johnson, 2010), plant and fungal species involved (Smith et al., 2004), and their competitive interactions (Näsholm et al., 2013; Hasselquist et al., 2016). Further, plants are simultaneously colonized by multiple fungi and fungi colonize multiple plants, forming complex networks. Given the substantial amount of resources moving between plants and fungi, and potential consequences for community and ecosystem ecology, it is important to understand the underlying mechanisms.

The flow of resources in mycorrhizal symbioses has been suggested to parallel those in markets, in which individuals profit by discriminating among potential trading partners based on the quality and quantity of the goods they offer. Biological Market Models (BMMs), or biological market theories, are evolutionary frameworks explaining the maintenance of the mycorrhizal symbiosis (Schwartz & Hoeksema, 1998; Wyatt et al., 2014). Trade is based on the relative value of goods or benefits provided by a partner (Noë & Kiers, 2018). The relative value is expressed as a 'price'. Because there is no common currency between plants and fungi, price is expressed as an 'exchange rate', for example the number of units of carbon per unit of nutrient (nitrogen or phosphorus). The actual price for a given exchange reflects a combination of dynamic factors such as supply and demand, and nutrient acquisition efficiencies of the partners. Although terms such as 'partner choice' and 'sanctions and rewards' have become synonymous with BMMs in some of our literature (Box 1), we suggest that, at their core, BMMs require that: (1) resources are exchanged between partners of different classes; (2) resources have a price, that is they differ in their relative value; and (3) prices

Box 1 Terms used to describe resource transfer between mycorrhizal symbionts

A biological market is a system that allows for the exchange of goods or services among two or more different trader classes (Noë & Kiers, 2018). Traders have commodities that differ in value or 'prices'. If prices do not respond to shifts in supply and demand, market mechanisms are absent (Noë & Kiers, 2018). Thus, at their core, markets are price-regulated.

Biological Market Models use economic principles to predict resource exchange between mycorrhizal fungi and plants (Johnson et al., 2006).

Comparative Advantage Model is a type of biological market model used to investigate the conditions under which species specialize and trade (Schwartz & Hoeksema, 1998). It is based on the concept of 'relative advantage', in which one partner is relatively more efficient at the production of one commodity than it is for another commodity.

'Sanctions and Rewards' is another type of biological market model in which resource exchange is based on reciprocity. Based on variation in the quantity or quality of resources, the complementary trader class chooses its partner. Prices for resources are set by outbidding competition among multiple partners within a trader class (Noë & Kiers, 2018). Reciprocity, that is the carbon transferred from plants based on nutrients delivered by fungi, requires an exchange mechanism to be present at the symbiotic interface.

Functional equilibrium is a concept that describes the dynamic distribution of dry matter between shoots and roots, where plants shift resource allocation towards shoot growth when growth is impaired by carbon acquisition and towards root growth when it is impaired by low levels of nutrients or water (Brouwer, 1963).

Partner choice is a one-time occurrence in advance of any possible exploitation (Bull & Rice, 1991), for instance, initial fungal colonization of the host plant. This term has also been used to incorporate 'sanctions' and 'rewards' as described below. Both partner choice and 'sanctions and rewards' have become synonymous with biological market models, however, these features define a single type of market model. We recommend distinct definitions for these terms to reflect the different mechanisms that would underlie each.

Sanctions are applied by 'cutting off the flow of a resource that is exchanged in trade' (Noë & Kiers, 2018). Rewards are given by enhancing the flow of said resource. Within biological market models applied to mycorrhizas, this is analogous to a plant directing more carbon toward a fungus which returns more phosphorus or nitrogen.

Reciprocal rewards/reciprocity occurs when partners deliver resources to each other in response to resources they receive. Reciprocity results in a bidirectional transfer of resources between plants and fungi.

Coupling results in a bidirectional transfer of resources between plants and fungi but is not in response to investment by the other partner.

Price in biological markets is the relative value of traded goods or services. The price for a given exchange reflects a combination of dynamic factors such as nutrient acquisition efficiencies of the partners and nutrient availability. Adjustment of prices can occur through competition among potential partners to be selected by the choosing class or through shifts in resource acquisition abilities and resource requirements of the partners.

Exchange rates express prices because there is no common currency between plants and fungi. In mycorrhizas, the exchange rate is the number of units of carbon against one unit of nutrient (usually nitrogen or phosphorus).

Surplus carbon is carbon that plants acquire beyond current demand and is typically higher when growth is nutrient-, water-, or temperature-limited. Surplus carbon is not to be confused with carbon accumulated in plants to the point it is harmful and requires removal. Whether carbon transferred to mycorrhizal fungi is in surplus of plant demand has so far been evaluated by measuring plant growth. However, a broader view of surplus carbon is one that recognizes all of a plant's carbon needs. Moving forward, this term should be defined as either 'carbon beyond what a plant can use for growth' or 'carbon beyond what a plant can use for any function'.



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Fig. 1 Historical frameworks of carbon transfer from arbuscular (AM) and ectomycorrhizal (ECM) plants to fungi represent a range of connections between carbon movement to fungi and nutrient movement to plants. The Surplus C Disposal Hypothesis states that the movement of carbon (black arrows) to the fungus follows source–sink dynamics and is no different than the movement of carbon toward other soil organisms or soil in general, that is nutrient delivery is not the basis for carbon transfer. The Surplus C Functional Equilibrium Hypothesis states that carbon moving to mycorrhizal fungi increases the ability of mycorrhizal fungi to return nutrients (brown arrows) which creates a feedback cycle with the transfers happening sequentially as indicated by arrows 1, 2, 3, 4. Under this hypothesis, the connection between carbon and nutrient transfer is indirect; carbon is drawn to fungal sinks and fungi transfer nutrients to plants but there is not direct reciprocity at the symbiotic interface. Importantly, in both Surplus C hypotheses, carbon transferred is not at the expense of plant growth, and the units of nutrient received per unit carbon transferred (i.e. the exchange rate) vary, but do not regulate exchanges. Finally, in Biological Market Models (BMMs) exchange rates play a fundamental role. Under Comparative Advantage BMMs they explain different outcomes of the symbiosis. Under Sanctions and Rewards BMMs carbon transfer depends on nutrient transfer, being higher or lower depending on exchange rates, or 'prices', of nutrients, and includes interactions with and among multiple potential partners, for example 'outbidding competition'. Thus, under the Sanctions and Rewards BMM, carbon transfer to the fungus is locally coregulated by the nutrient supply of the fungus, whereas Surplus C hypotheses propose that carbon transfer is regulated by plant stoichiometry and carbon sink strength, and only dependent on fungal nutrient supply insofar as it affects stoichiometry and carbon sink strength.

determine outcomes and/or regulate the exchanges. Biological market models make no predictions about the mechanisms by which resource exchange occurs, but exchanges are generally mutualistic.

This view has been disputed, and it has been proposed that carbon transfer is independent of the amount of nutrients received (Corrêa *et al.*, 2012; Prescott *et al.*, 2020; Prescott, 2022). Under this view, the carbon transferred to mycorrhizal fungi is in surplus to what can be used for growth due to nutrient limitations. Consequently, plant growth responses to mycorrhizas depend on the amount of nutrients received from the fungi and cannot be negatively affected by carbon transferred to the fungi (Corrêa *et al.*, 2012, 2023; Horning *et al.*, 2023). The BMMs and what we refer to as 'Surplus C hypotheses' (but see Reconsidering the term 'surplus-

carbon' below) attempt to explain different phenomena (the maintenance of mycorrhizal symbioses vs the physiological mechanisms regulating resource flow, respectively), and as such, they are not entirely comparable. However, at the core of each framework are fundamental, and contrasting, assumptions about the regulation of resource exchange (Fig. 1), and what explains mycorrhizal growth responses (Table 1). Specifically, transfer of carbon is optimized relative to nutrient delivery by the fungi in BMMs, and while BMMs can incorporate the exchange of surplus resources (Noë, 2021), they often assume plant growth suppressions result from costly carbon transfer to the fungus (reviewed in Horning *et al.*, 2023). Contrarily, under Surplus C hypotheses, carbon transfer is a metabolic consequence of the nutritional status of plants and without regulation mechanisms ensuring optimal exchange rates. Resolving these different

Frameworks		Motivation	Factor regulating carbon transfer	Carbon and nutrient transfer locally co-regulated?	Theoretical or conceptual basis
Biological Market Models	'Sanctions and rewards'	Explain cooperation between species	Based on economic principles Plant investment is in response to investment by the fungal partner: 'reciprocity'	Yes	Kiers & Denison (2008)
	'Comparative advantage'	Investigate the conditions under which plants specialize and trade with fungi Investigate outcomes of plant and fungal interactions (mutualism to parasitism)	Based on economic principles	Not specified	Schwartz & Hoeksema (1998); Johnson (2010)
Surplus C Hypotheses	'Surplus C Functional Equilibrium'	Investigate outcomes of plant and fungal interactions (mutualism to parasitism)	Maintain functional equilibrium between shoots and mycorrhizal roots Carbon in surplus for plant growth is allocated to mycorrhizal fungi	Q	Corrêa et al. (2012, 2023)
	'Carbon Disposal'	Provide a non-adaptive view of the transfer of carbon to fungi	Disposal; plant has more carbon than needed for growth	No	Prescott et al. (2020)

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charge rates). Surplus C Hypotheses posit that carbon flow is a metabolic consequence of the nutritional status of plants and is directed by source-sink dynamics, independent of local delivery of nutrients, that is exchange rates play no role in either regulation or the outcome of exchanges NUVAIILAKE Sanctions and Rewards' they regulate the trade, when

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perspectives is key to understanding interactions between plants and mycorrhizal fungi to better predict conditions where mycorrhizal fungi may promote plant growth. Focusing on AM and EcM where most research has been done, we: (1) introduce the reader to these disparate frameworks, their history and some points of criticism; (2) examine support for either framework in the literature; and (3) present new ideas and future experiments.

1. History of the Biological Market Models and Surplus C hypotheses

Biological Market Models Biological Market Models were first developed to understand cooperation between organisms. Emerging from the Prisoner's Dilemma game (Axelrod & Hamilton, 1981), which explored the strategic behavior between two individuals, BMMs added partner choice and introduced the idea that partners monitor markets (Noë & Hammerstein, 1994). These models predict an evolutionary advantage for partners that specialize in acquisition of, and/or preference for, different resources, so long as they trade with each other for the other resource (Schwartz & Hoeksema, 1998). If cooperation can be enforced, the exchange of resources can benefit each of the partners and stabilize into a mutualism (Noë & Hammerstein, 1995). Thus, these models offer a possible explanation for the evolutionary stability of mycorrhizas, where plants and fungi vary in their ability to acquire essential resources.

Two versions of BMMs were developed to better understand mycorrhizal function and outcomes of partner interactions: (1) 'Comparative Advantage' (e.g. Schwartz & Hoeksema, 1998; Johnson, 2010); and (2) 'Sanctions and Rewards' (e.g. Kiers & Denison, 2008) (Table 1). 'Comparative Advantage' models predict outcomes (more or less beneficial) of mycorrhizal interactions based on resource exchange rates (Schwartz & Hoeksema, 1998; Hoeksema & Schwartz, 2003; Johnson et al., 2006; Johnson, 2010). Different exchange rates, or prices, will result from variations in plant and fungal resource acquisition abilities (both inherent and depending on environmental factors) and resource requirements to maintain metabolic stoichiometry within partners (Schwartz & Hoeksema, 1998; Hoeksema & Schwartz, 2003; Kummel & Salant, 2006; Johnson, 2010). By considering both acquisition ability and resource requirements, these models predict conditions where it is beneficial for plants to trade carbon for nutrients with mycorrhizal fungi vs acquiring nutrients directly.

The second type of BMM, 'Sanctions and Rewards', centers on partner choice (Werner et al., 2014; Werner & Kiers, 2014) where multiple competing partners within a trader class enable outbidding for resources offered (Noë & Hammerstein, 1994). Based on variation in the quantity or quality of goods, the complementary trader class chooses its partner. The price is dynamic and an outcome of the exchange, but the exchange itself is regulated by transfer of goods that differ in value. Applied to mycorrhizas, a plant rewards fungi that deliver more nitrogen or phosphorus by supplying more carbon, and sanctions fungi that deliver less nutrients (Axelrod & Hamilton, 1981). Partner choice was initially described as a

one-time occurrence in advance of any possible exploitation (Bull & Rice, 1991), for instance, initial fungal colonization of the host plant. However, cooperation between mycorrhizal partners has rarely been investigated as a one-time occurrence, but instead as on-going sanctions and rewards to established symbionts (Denison, 2000; Kiers & Denison, 2008; Kiers et al., 2011). Although Noë & Kiers (2018) suggested that partner choice include 'any form of discrimination among actual and potential partners at any stage of the interaction', potential partner discrimination requires recognition, whereas actual partner discrimination involves responding to partner behavior in established mycorrhizas. For these reasons, we use the original definition of the term 'partner choice' to describe discrimination among potential partners, and the term 'sanctions and rewards' to discuss discrimination among actual partners. Critical to the Sanctions and Rewards BMM is that resource exchange is based on reciprocity whereby partners deliver resources to each other in response to resources they receive in a game of 'Tit for Tat' (Axelrod & Hamilton, 1981; Kiers & Denison, 2008) (Table 1).

Biological market models have received some criticisms, mostly directed at the Sanctions and Rewards BMM and its underlying concepts. For example, Walder & van der Heijden (2015) argued that reciprocal rewards and market dynamics are relatively unimportant for driving resource exchange because of the existence of parasitism, lack of specificity, common mycorrhizal networks precluding monitoring of resource supply, and partner identity controlling resource exchanges rather than reciprocity. In response, Kiers et al. (2016) pointed out that tightly coupled resource exchanges are not assumed in BMMs, cheating can be accommodated, low partner specificity facilitates the evolution of stable trading, variation in the precision of partner choice is expected, and variable rewards and changing partner preferences are the defining features of biological markets. Other issues raised with the Sanctions and Rewards BMM include its broadly permissive outcomes, which makes development of testable hypotheses difficult (Smith & Smith, 2015; van der Heijden & Walder, 2016), and whether the carbon exchanged is costly to plants (van der Heijden & Walder, 2016; Prescott et al., 2020 but see Noë, 2021). Most BMMs involve trading surplus resources (Noë & Hammerstein, 1995; Schwartz & Hoeksema, 1998; Kiers & van der Heijden, 2006, but see Kummel & Salant, 2006). Yet, BMMs allow for 'cheaters' where fungi take carbon without returning nutrients, which in effect are parasitic interactions (Hoeksema & Bruna, 2000) that fit with earlier suggestions of parasitic mycorrhizal fungi (Johnson et al., 1997). These suggestions combined with suppressed growth of mycorrhizal plants spawned the current tenet that the balance between nutrient benefit received and carbon expended explains plant growth responses (e.g. Johnson et al., 1997). The underlying assumption here is that carbon availability can limit plant growth, and growth depressions occur in plants when the carbon transferred to the fungal partner would otherwise have been used for plant growth. Because BMMs require price regulation, determining whether carbon transferred to mycorrhizal fungi would have otherwise been used for plant growth has implications for market prices. If price regulation is disproven, then biological markets cease to exist.

Surplus C Unlike BMMs, which have theoretical origins, Surplus C hypotheses grew from experimental evidence and are not based on economic principles (Kaschuk *et al.*, 2009, 2010; Corrêa *et al.*, 2012). Surplus C hypotheses rely on limited observations that plant growth is more sensitive than photosynthesis to reductions in water and nutrients (Hsiao, 1973; Muller *et al.*, 2011). Therefore, under mild-to-moderate water and nutrient deficiencies, which is common, plants are thought to produce 'surplus-carbon', or carbon that cannot be used for growth (Körner, 2015). This leads to increased carbon export to sinks such as roots and their associated mycorrhizal fungi (Prescott *et al.*, 2020). Though recently proposed, this idea has been around since at least 1970 (Björkman, 1970).

We identified two main hypotheses under Surplus C, which we titled: 'Surplus C Functional Equilibrium' and 'Surplus C Disposal' (Table 1). Together, these hypotheses differ from BMMs in that: (1) carbon transferred to fungi is never costly and will not cause negative effects on plant growth or other functions; (2) the amount of carbon transferred to mycorrhizal fungi depends on plant metabolism and fungal carbon sink strength, and is independent of nutrient return from the fungus.

The Surplus C Functional Equilibrium Hypothesis follows the concept of 'functional equilibrium' (Brouwer, 1963), which states that plants shift resource allocation toward shoots if carbon acquisition is impaired by low light or CO2, and toward roots when nutrients and/or water are limiting (Fig. 1). These shifts are adaptive and enable plants to capture more of the resources that most strongly limit plant growth (Poorter & Nagel, 2000). The Surplus C Functional Equilibrium Hypothesis extends this concept by considering mycorrhizal fungi as a functional extension of roots. This hypothesis is informed by the observation that transfer of carbon to mycorrhizal fungi depends primarily on plant nutrient status - not nutrient uptake - (2), and secondarily on fungal carbon sink strength in ways consistent with mechanisms already described for non-mycorrhizal plants (Corrêa et al., 2023). Thus, carbon transfer can be understood even in the absence of fungi, that is the interacting trader class, which is a key feature of BMMs. Furthermore, although an increased distribution of carbon to roots in low nutrient conditions is accommodated by all frameworks, the Surplus C Functional Equilibrium Hypothesis considers it a sufficient mechanism explaining carbon transfer to the fungal partner. As such, the Surplus C Functional Equilibrium Hypothesis integrates sourcesink dynamics within and between organisms, which agrees with the view recently presented by Bogar (2023). The functional equilibrium concept has been applied to mycorrhizas previously (Johnson et al., 2003, 2006; Johnson, 2010) but the Surplus C Functional Equilibrium Hypothesis differs from other proposals because it does not consider carbon transfer to the fungal partner to be responsible for growth depressions in mycorrhizal plants.

Similar to the Surplus C Functional Equilibrium Hypothesis, Prescott and colleagues (Prescott *et al.*, 2020; Prescott, 2022) proposed that carbon transfer to mycorrhizal fungi is driven by the production of 'surplus-carbon'. But in contrast to Surplus C Functional Equilibrium, carbon transfer reflects the plant's disposal of surplus-carbon to avoid feedback inhibition of photosynthesis and photodamage, rather than promoting nutrient delivery from the fungal partner. Thus, under the Surplus C Disposal Hypothesis, mycorrhizal fungi serve only as carbon sinks, and any increased nutrient delivery is coincidental (Fig. 1).

While both Surplus C hypotheses are new and have not yet received much attention by mycorrhizal researchers (Supporting Information Methods S1), plant physiologists have argued whether carbon availability limits plant growth, particularly in long-lived trees (Sala et al., 2012; Wiley & Helliker, 2012). Further, whether carbon transferred to fungi is in surplus for plant functioning has so far been evaluated by effects on plant growth only, without considering competing sinks including storage of nonstructural carbohydrates (Blumstein et al., 2022) and lipids, which can increase stress tolerance and survival (O'Brien et al., 2014; Sapes et al., 2021). This raises the question of whether mycorrhizal effects on carbohydrate storage (Sapes et al., 2021) may lead to negative effects on survival and growth in the long term that are not accounted for when examining only short-term plant growth. Some have also argued that it is difficult to reconcile Surplus C Disposal with the evolution of multiple traits facilitating partner choice and trading (Noë, 2021). In response, Prescott et al. (2020) state their hypothesis does not preclude these types of adaptations. If, however, carbon and nutrient transfer is coregulated at the local level, then Surplus C hypotheses would be rejected as proximate explanations for carbon transfer. Further, if fungal parasitism can be demonstrated, then Surplus C would not hold.

II. Empirical evidence for and against BMMs and Surplus C hypotheses

Biological market models have often been tested in physiological experiments and considered supported when plants deliver more carbon to fungi that deliver more nutrients to plants (e.g. Kiers et al., 2011). However, higher nutrient availability may coincide with more active mycorrhizas and greater carbon sinks (Corrêa et al., 2023). Thus, experimental results interpreted as supporting BMMs could also be interpreted as supporting Surplus C hypotheses. We next reviewed experiments performed at the organismal and cellular level with three main goals: (1) summarize the framing and the findings of studies that asked whether plants transfer more carbon to fungi that transfer more nutrients (consistent with Sanctions and Rewards BMMs); (2) quantitatively assess whether growth suppressions by mycorrhizal fungi are related to carbon (inconsistent with Surplus C hypotheses); and (3) review the current understanding of cellular mechanisms for carbon transfer from plants to fungi and whether it is regulated by nutrient delivery from fungi (consistent with the Sanctions and Rewards BMM).

1. Can plants selectively reward fungal partners?

Mycorrhizal plants are often colonized by multiple fungal species that vary in the amount of nutrients they deliver (e.g. Smith

et al., 2003; Albarracín et al., 2013). Under the Sanctions and Rewards BMM, plants transfer more carbon to fungi that deliver more nutrients (Kiers et al., 2011). Contrarily, in Surplus C hypotheses, carbon moves to the strongest sink, irrespective of nutrient delivery (Corrêa et al., 2023; Table 1). We searched the literature to assess the extent to which plants transfer more carbon to mycorrhizal fungi that transfer more nutrients, and whether those fungi may also represent the largest carbon sink (see Methods S1 for search details and selection of papers). Specifically, we examined studies where plants had been inoculated with two or more fungal taxa, where fungal symbiont 'quality' had been tracked using plant biomass, phosphorus isotopes, or nitrogen isotopes, and where carbon transfer to fungi had been assessed using either fungal biomass (root colonization, spore biovolume, or quantitative polymerase chain reaction) or concentration of ${}^{13}C$ or ${}^{14}C$ isotopes in fungal tissue. We did not include papers that modified resource availability on single taxa because more nutrients would likely be accompanied by higher fungal metabolism. As such, a potential increase in carbon transfer could simply be a consequence of greater sink strength, rather than a 'reward'. For this reason, comparisons were always made between different fungal taxa at the same resource level colonizing the same plant. We found 13 papers that included multiple taxa (9 AM and 4 EcM), with some containing several trials where fungi were either physically separated or mixed, or where taxa were compared at different resource levels (Table S1).

We found that 10 papers used the BMM framework to introduce the study as opposed to concepts associated with Surplus C hypotheses (one paper; two papers were ambiguous; Table S1). We also found an almost even split between trials where plants delivered more carbon to fungal partners that promoted more growth or delivered more nutrients (eight trials), and where they did not (seven trials). In three trials, fungi did not differ in the amount of nutrients they delivered, and in four trials, we could not assess whether transfer differed as no direct comparisons between treatments were made or the significance of the difference between treatments was uncertain. Whether or not plants preferentially transfer carbon to the largest sink was unknown in most studies as only some carbon pools (e.g. root colonization or spore biovolume, or quantitative polymerase chain reaction in roots) were accounted for.

This exercise highlights several important points. First, most studies have used BMMs as a guiding framework to study preferential transfer of carbon among competing mycorrhizal fungi (Table S1). Second, plants do not appear to be universally capable of identifying and selectively transferring more carbon to fungi that transfer more nutrients, at least not in the highly controlled conditions used in most of these experiments. This lack of support for BMMs, however, does not necessarily mean support for Surplus C hypotheses. Third, transfer of carbon to the fungus delivering more nutrients could not be separated from the possibility that this was also the largest and/or more active symbiont and therefore the greatest carbon sink. Quantifying fungal sink strength would require researchers to measure all fungal carbon pools, including respiration, which was not done in any of the studies, quite understandably as that is a monumental task. To distinguish

support for BMMs from support for Surplus C, both sink strength and nutrient delivery need to be quantified as they may coincide. Fourth, three studies (Argüello et al., 2016; Hortal et al., 2017; Bogar et al., 2022) showed that fungi increased nutrient transfer when co-occurring with fungal taxa that delivered more nutrients than when they occurred by themselves. Thus, the amount of nutrients transferred by a single taxon is not static but depends on the presence of other taxa. Several papers (Zheng et al., 2015; Ji & Bever, 2016; Werner et al., 2018; Bogar et al., 2022) also showed that preferential transfer depends on resource availability, further adding to its context dependency. Finally, colonization by the least cooperative partner may be limited by an upregulation of defense-related genes, not by a reduced carbon transfer to the fungus (Hortal et al., 2017). This agrees with recent arguments put forth by Bogar (2023) and highlights that many additional factors contribute to the nonrandom distribution of fungal taxa often found among plant species, including fungal competition (Bever et al., 2009; Kennedy et al., 2009) and plant and fungal spatial co-occurrences (Kokkoris et al., 2020).

2. Are mycorrhizal growth depressions in plants tied to carbon transfer?

Manipulating carbon availability in mycorrhizal plants may help us better understand carbon transfer to fungi. Indirect methods for controlling carbon availability include modifying CO2 concentrations and/or reducing light through shading. An earlier meta-analysis showed elevated CO2 concentrations result in long-term and often substantial increases in fungal biomass (Treseder, 2004). This finding indicates that fungal growth is generally limited by carbon delivery from plants, but it does not distinguish between BMMs and Surplus C hypotheses as both would predict more carbon transfer to fungi with increasing nutrient deficiency. However, BMMs accommodate fungal parasitism whereas Surplus C hypotheses do not, as the latter predicts that no carbon is transferred to the fungus that the plant could otherwise use. In other words, plant growth cannot be depressed by carbon transfer. If growth of mycorrhizal plants is reduced more than nonmycorrhizal plants when shaded, we could infer that carbon transfer to mycorrhizal fungi is at the expense of plant growth. To test this prediction, we extracted data from studies that manipulated light availability and measured plant and mycorrhizal fungal growth responses. Using these data, we conducted three meta-analyses to determine whether: (1) plant growth; and/or (2) fungal growth was generally reduced by shading treatments; and (3) if mycorrhizal plants differed in biomass from nonmycorrhizal plants when shaded.

We extracted data on plant and mycorrhizal fungal biomass and potential covariates from 43 papers (37 AM and 6 EcM) where shading ranged from 10% to 98% reduction of ambient light (Methods S2). Most plant biomass responses were either total biomass or shoot biomass, but a few used proxy measurements such as height and leaf area (Table S2). Most fungal biomass responses were root colonization, although we prioritized spore abundance where available as it is less affected by changes in plant biomass (Table S2). Covariates included the intensity and duration of Tansley review



Fig. 2 Effect of shade on plant and fungal biomass for (a) mycorrhizal vs nonmycorrhizal plants, (b) arbuscular- (AM) vs ectomycorrhizal (EcM) fungi. Estimates are below the line of no effect (y = 1), indicating that plant and fungal biomass is generally reduced with shading. Lines indicate 95% confidence intervals. Some confidence intervals are asymmetrical because values were back-calculated from the log response ratio which was used in the meta-analyses. Replication at three hierarchical levels is shown on each graph and dependencies among these levels were accounted for in our models. Data characteristics and details of the analysis are included in Supporting Information Methods S2.

shading, location of study, and fertilizer applications as they can all affect plant biomass responses (e.g. Smith & Gianinazzi-Pearson, 1990; Whitbeck, 2001; Konvalinková & Jansa, 2016). Additional details are in SI including data extraction, handling, and analysis (Methods S2), biomass and biomass proxy variables and replication (Table S2), covariates and replication (Table S3), results (Table S4), and data (Tables S5–S7).

Overall, we found that plant biomass was suppressed in shade whether or not plants were mycorrhizal, indicating that shading reduced photosynthesis to the point that carbon was limiting for plant growth (Fig. 2a; Table S4). Shade also reduced fungal biomass (Fig. 2b; Table S4). Although we found larger biomass reductions in EcM than AM fungi, replication of EcM studies was low and we place more emphasis on the direction than the magnitude of the effect. The consistent trend of biomass reduction suggests a reduced carbon transfer to mycorrhizal fungi, which agrees with Konvalinková & Jansa (2016).

Importantly, mycorrhizal plants grew more than nonmycorrhizal plants under shade (Fig. 3; Table S4). Thus, at least in these



Fig. 3 Effect of mycorrhizas on plant biomass with fertilization (circles) and without (squares) in (a) ambient conditions (yellow), and (b) with shade (grey). In general, mycorrhizas increased plant biomass in both ambient and shaded conditions as evidenced by the estimates for unfertilized treatments, but this effect disappeared when fertilizer was added. Lines indicate 95% confidence intervals. Some confidence intervals are asymmetrical because values were back-calculated from the log response ratio which was used in the meta-analyses. Data were primarily from AM studies and replication at three hierarchical levels is shown on each graph and dependencies among these levels were accounted for in our model. Data characteristics and details of the analysis are included in Supporting Information Methods S2.

experiments, which were primarily AM studies (Table S3), carbon transfer to fungal symbionts did not affect plant growth even under conditions of carbon limitation. Furthermore, the mycorrhizal effect disappeared when plants were fertilized (Fig. 3; Table S4), fitting with other work where growth responses correlated with nutrient delivered to plants, not carbon delivered to mycorrhizal fungi (Corrêa et al., 2012, 2023; Horning et al., 2023). Overall, our results are consistent with the Surplus C Functional Equilibrium hypothesis because carbon transferred to fungi did not come at the expense of plant biomass, and plant growth responses were dependent on nutrient transfer from the fungi. However, BMMs accommodation of parasitism is not a requirement, thus BMMs cannot be rejected based on these results alone.

3. Is transport of carbon across the symbiotic interface linked to nutrient supply?

A core difference between Sanctions and Rewards BMMs and Surplus C hypotheses is that reciprocal rewards regulate resource exchange in the former and source-sink gradients drive carbon distribution in the later. Thus, Sanctions and Rewards BMMs imply that carbon transport to the fungus is locally regulated by the nutrient supply of the fungus, whereas Surplus C hypotheses propose that carbon transfer is regulated by plant stoichiometry and carbon sink strength, and only depend on fungal nutrient supply insofar as it affects stoichiometry and carbon sink strength. We next outline what is currently known about regulation mechanisms involving transporters that transfer sugar and fatty acids from root cortical cells into the apoplast where mycorrhizal fungi occur. Because the study of mycorrhiza-specific or mycorrhiza-regulated

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membrane transporters is in its early stages, our conclusions are tentative.

Sugar transport to the symbiotic interface The presence of mycorrhizal fungi often increases root carbon sink strength in both AM and EcM plants (Cairney et al., 1989; Wu et al., 2002; Gavito et al., 2019), increasing transport of sugars belowground. To become available to fungi, sugars must be transported across plant cell membranes into the apoplast/symbiotic interface, which typically involves transporter proteins (Fig. 4). In AM plants, some sugar transporters are induced by mycorrhizas (Harrison, 1996; Boldt et al., 2011; Gaude et al., 2012; Manck-Götzenberger & Requena, 2016; An et al., 2019). Those that have been characterized are facilitated transport channels called Sugars Will Eventually be Exported Transporters (SWEETs; Manck-Götzenberger & Requena, 2016, An et al., 2019). Transport into the apoplast/symbiotic interface in AM may therefore be passive and respond to decreasing sugar concentrations in the apoplast resulting from fungal uptake. In EcM plants, expression of a SWEET1 is also upregulated in oak mycorrhizas (Tarkka et al., 2013), indicating that the same may be true for EcM. Other plant sugar transporters - most of which have homology to active H+ symporters (Nehls et al., 2000; Grunze et al., 2004) or were not characterized (Wright et al., 2000; Tarkka et al., 2013) - were found to respond to mycorrhizas, but mostly through downregulation (Nehls et al., 2000; Wright et al., 2000; Grunze et al., 2004) showing decreased active transport. Exceptions are the plant hexose transporter PttMST3.1 (EcM; Grunze et al., 2004) and sucrose transporter SISUT2 (AM; Bitterlich et al., 2014) where expression increased with mycorrhiza formation. This has, however, been implicated in sugar reuptake from the apoplast (a topic we revisit below), rather than transport into the apoplast.

In contrast to plant sugar transporters, all fungal sugar transporters that have been identified in AM and EcM are active H+ symporters (Nehls et al., 1998, 2001; Wiese et al., 2000; Fajardo López et al., 2008; Helber et al., 2011; Ceccaroli et al., 2015; Lahmidi et al., 2016). Thus, sugars seem to move out of plant cells into the apoplast passively where they are actively taken up against concentration gradients by fungi. The continued flow of sugars into the apoplast may therefore depend on the speed by which fungi are able to acquire them.

Regulation of sugar transporters is only partially understood (Chen et al., 2015), but we know they can be regulated by sink strength (Büttner & Sauer, 2000) and sugar concentrations (Caspari et al., 1994; Bush, 2004), which is consistent with Surplus C hypotheses. We did not find any reports of direct regulation of sugar transporters by nutrients, which would support Sanctions and Rewards BMM, and there is even some indirect evidence suggesting sugar transport is independent of nutrient transport. For example, knockdown of the mycorrhiza-induced plant NH4+ transporter AMT3;1 decreased uptake of 15N from AM fungi but did not affect mycorrhiza formation (Koegel et al., 2017), suggesting no change in carbon delivery from plants to fungi. Likewise, fungal colonization was not affected by mutations in the plant H+-ATPase gene MtHA1, which reduced phosphate uptake at the periarbuscular membrane (Hubberten et al., 2015).

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Fig. 4 Current knowledge of transporters, substrates and possible points of regulation related to sugar and fatty acid transport in mycorrhiza symbiotic interfaces. Possible points of regulation: 1. Sucrose (suc) cleavage. Suc must be cleaved into fructose (fru) and glucose (glu) before uptake by mycorrhizal fungi. This can be performed (1a) inside the plant cell; in the cytoplasm by sucrose synthase (SuSy) or neutral invertase (NI), or in the vacuole by vacuolar acid invertase (Alv), or (1b) after sucrose transport into the apoplast, by cell wall acid invertase (Alcw). Increased SuSy, NI, and Alv activity have been found in mycorrhizal plants. 2. Sugar transport into the apoplast. Suc or hexoses (fru, glu) are transported from plant cells into the apoplast through channels (SWEETs - passive transport). 3. Sugar transport into the fungal cell. Glu is preferentially taken up by mycorrhizal fungi through H+ coupled symporters (active transport). Glu uptake decreases glu concentrations in the apoplast, maintaining the concentration gradient between plant cytoplasm and apoplast and driving further glu transport into the apoplast. 4. ATP-driven H+ pumping. Sugar transport into the fungal cell and nutrient transport into the plant cell are driven by membrane potential ($\Delta\Psi$) and transmembrane pH gradients (Δ pH). These are created by (4a) fungal and (4b) plant H + \cdot ATPases, which use the energy of ATP hydrolysis to translocate protons from the cytoplasm to the extracellular space. 5. Sugar reuptake into plant cells. Upregulation of a hexose H+ symporter in ectomycorrhizas (EcM) may indicate fru reuptake into plant cells. Fru reuptake would prevent fru accumulation due to preferential glu uptake by the fungus, and associated feedback inhibition of Alcw activity. An upregulated suc transporter in arbuscular mycorrhizas (AM) may play a role in maintaining sugar supply to the fungus in check. Other, uncharacterized upregulated plant transporters may serve similar functions. 6. Fatty acid transport. In AM, fatty acids are transported (6a) into the apoplast by plant ABC transporters, and (6b) taken into fungal cells by fatty acid transporters. 7. Target of rapamycin (TOR): TOR is a central regulatory hub integrating multiple signals. Namely, it is activated by nutrient and glu concentrations. In AM plants, it was observed to regulate the expression of SWEETs and genes involved in fatty acid supply to AM fungi.

Although sugar transporters appear independent of nutrients, nutrient transporters are induced by sugars (Lejay *et al.*, 1999, 2003). This seems to also apply to AM-specific phosphorus transporters, in both plants and fungi (Xie *et al.*, 2016). Thus, while studies to date do not indicate that carbon delivery from plants requires nutrient delivery by fungi as predicted by Sanctions and Rewards BMM, nutrient delivery by fungi may depend on carbon transfer from plants. More studies assessing differential expression of plant sugar transporters in response to nutrient supply by fungi, or indirect regulation through signaling, are needed.

Instead of regulating sugar transport into the apoplast, plants may regulate sugar concentrations in the apoplast through reuptake. For instance, plants may compete with fungi for hexoses in the apoplast when nutrient delivery from the fungus is low, which would link regulation to nutrients transferred from fungi. Induction of plant hexose transporter PttMST3.1 in EcM roots has been considered evidence of such regulation (Grunze *et al.*, 2004), but a link between PttMST3.1 and nutrient concentrations was not tested. A contrasting possibility, which would maintain rather than restrict sugar supply to the fungus, is that plants may reuptake fructose to prevent accumulation in the apoplast (resulting from preferential glucose uptake by the fungus) and feedback inhibition of AIcw (see *Sucrose cleavage into glucose and fructose*). However, this would only be relevant if AIcw is the main enzyme responsible for glucose supply to the fungus. In AM, a permanent, rather than nutrient-related, mechanism to regulate sugar supply to the fungus has been suggested. The tomato sucrose transporter SISUT2 is induced in the periarbuscular membrane of AM roots and may retrieve sucrose from the symbiotic interface. Its downregulation in roots of antisense mutants increased mycorrhiza formation (Bitterlich *et al.*, 2014), suggesting fungi have access to more sugar when this transporter is absent.

In addition to sugars, fatty acids are a major carbon form transferred to AM fungi (Bell et al., 2024), and AM fungi depend on their host plant for fatty acid synthesis (Bravo et al., 2017; Jiang et al., 2017; Luginbuehl et al., 2017). Fatty acid transport can occur through ABC transporters, and heterodimeric ABC transporters have been identified in Medicago truncatula and Oryza sativa that are necessary for arbuscule formation (Zhang et al., 2010; Gutjahr et al., 2012; Jiang et al., 2017). ABC transporters are directly energized by ATP, indicating that in contrast to sugar transport, at least some fatty acid transport into the apoplast is active. As such, and because AM fungi cannot produce fatty acids, this could be one way plants regulate carbon movement to the fungus (and restrict carbon movement to herbivores and pathogens; Bell et al., 2024). However, mycorrhizas still form in mutant plants with disrupted fatty acid production and transport, provided that the fungi simultaneously colonize a wild-type plant (Gutjahr et al., 2012; Jiang et al., 2017). Thus, ceasing the transfer of fatty acids does not prevent mycorrhiza formation if the fungus can obtain fatty acids from other plants within a mycorrhizal network. Future research should assess whether active fatty acid transport is regulated by nutrients, which would support Sanctions and Rewards BMMs.

Sucrose cleavage into glucose and fructose Mycorrhizal fungi are either unable or have a very limited ability to use sucrose, which is the main form of carbon transported within plants, and sucrose cleavage into hexoses (i.e. glucose and fructose) is necessary for sugar supply to fungi (Solaiman & Saito, 1997; Nehls et al., 2007; Miyauchi et al., 2020). Cleavage can be performed by plant sucrose synthase, cytosolic (neutral) invertase, vacuolar acid invertase, or cell wall invertase (AIcw), and these sucrolytic enzymes are necessary for mycorrhizas to form (Schaarschmidt et al., 2007; Baier et al., 2010). Because AIcw is in the apoplast, it could be the main enzyme responsible for supplying hexoses to fungi (Nehls et al., 2007). AIcw has optimal activity at pH c. 4, and the apoplast is acidified by mycorrhizal formation. This acidification can be partly due to H+ export by plant cells, necessary for plant nutrient uptake through H+ coupled symporters (McDonald et al., 2022). As such, it creates a possible mechanism linking carbon supply to fungi with nutrient uptake by the plant. On the other hand, increased apoplast acidification could be unrelated to nutrient uptake by plants and instead result from H+ export by fungal cells associated with hexose uptake (Fig. 4). Furthermore, AIcw activity does not change in response to EcM fungi (Hampp et al., 1995; Schaeffer et al., 1995; Wright et al., 2000; Corrêa et al., 2011), and enzymes other than Alcw are often more important for sucrolytic activity in AM (Blee & Anderson, 1998; Schellenbaum et al., 1998; Wright et al., 1998; Hohnjec et al., 2003; Ravnskov et al., 2003; Schubert et al., 2003; Schaarschmidt et al., 2006; García-Rodríguez et al., 2007). Thus, while still a possibility to be explored, there is currently little support that increased activity of AIcw due to apoplast acidification is an important mechanism linking hexose transfer from plants to fungi to nutrient uptake by the plant.

Another possible regulation mechanism derives from the fact that EcM and AM fungi preferentially take up glucose over fructose

(Fajardo López *et al.*, 2008; Roth & Paszkowski, 2017). This could lead to fructose accumulation in the apoplast and inhibition of Alcw, because sucrolytic enzymes are inhibited by fructose (Šebková *et al.*, 1995). If this is the case, then sucrose cleavage is regulated by fungal hexose uptake rather than nutrient delivery, with AIcw activity being inhibited with increasing sugar uptake by the fungal cells.

Indirect or upstream regulation of carbon metabolism and nutrient transport Carbon and nitrogen metabolisms are intimately connected and coregulated (Gutiérrez et al., 2007; Schachtman & Shin, 2007; Wang & Ruan, 2015). The target of rapamycin (TOR) kinase, a highly conserved enzyme found in all eukaryotes, is a key regulator of growth by receiving and integrating metabolic processes and environmental cues, including nutrient and sugar concentrations. When conditions are favorable for growth, TOR is active and promotes anabolic processes to drive growth while repressing catabolic processes. But when nutrients are limited or environmental stresses are present, TOR is inactivated, and catabolic processes are promoted (Burkart & Brandizzi, 2021). TOR signaling acts at a local level and may play a role in symbiotic relationships (Burkart & Brandizzi, 2021). Indeed, TOR activity increased in AM root cortical cells, whereas TOR knockdown mutants had stunted arbuscules, altered expression of sugar metabolism and SuSy, NI, and SWEETs genes, as well as plant genes involved in fatty acid supply to AM fungi (RAM2) and AM interactions (Arthikala et al., 2021). Integration of nutrient uptake and carbon flux to fungi may therefore be mediated by TOR and deserves further investigation because it could be a link between carbon and nutrient movement.

Mycorrhizal fungi may also affect carbon sink strength through hormones or effectors (signaling molecules) independently of nutrient supply. For example, carbon sink strength in plants can be affected by cytokinins and auxins-IAA (Wang & Ruan, 2015) and mycorrhizal fungi are known to produce plant hormones, including cytokinins, GABA, IAA, and ethylene (Blee & Anderson, 1998; Pons et al., 2020). Hormone production by AM fungi has also been hypothesized to be involved in stimulating invertase expression and activity in plant cells (Blee & Anderson, 1998). Regarding effectors, pathogens are known to manipulate plant sugar transport through effector proteins by activating the transcription of SWEETs (Chen et al., 2010; Aparicio Chacón et al., 2023). Effector proteins are also produced by AM fungi, which may suppress the plant immune response associated with colonization (Yi & Valent, 2013; Aparicio Chacón et al., 2023). Most AM fungal effectors have unknown functions, but effects on SWEETs and other transporters/enzymes involved in phloem unloading and sugar transport and catabolism cannot be excluded.

Other considerations Surplus C hypotheses propose that carbon transfer will use the same mechanisms as non-mycorrhizal plants. Thus, if the same plant sugar transporters are active in mycorrhizal and nonmycorrhizal roots and respond to the same stimuli, this could be evidence in support of the Surplus C hypotheses. Indeed, while several mycorrhiza-specific phosphate and nitrogen transporters exist (Guether *et al.*, 2009; Koegel *et al.*, 2013; Xie

et al., 2013, 2016), mycorrhiza-induced plant sugar transporters do not appear to be mycorrhiza-specific as they are expressed in other plant tissues and nonmycorrhizal areas of the root (Harrison, 1996; Nehls et al., 2000; Wright et al., 2000; Grunze et al., 2004; Boldt et al., 2011; Gaude et al., 2012; Garcia et al., 2016; Manck-Götzenberger & Requena, 2016; An et al., 2019). Yet, even without mycorrhiza-specific transporters, plants and fungi share a low-volume apoplastic space, allowing efficient acidification by plant or fungi H+ pumps. This may facilitate sucrose cleaving enzyme activity, as well as active uptake of resources by both plant and fungi via H+ symporters. The fact that plants and fungi share the same H+ gradient may represent a potential mechanism for coupled C and nutrient transfer across the interface: a high apoplastic H+ concentration leads to a high H+ gradient between apoplast and cytoplasm, which opens resource flow in both directions, conversely a low H+ concentration shuts down both (McDonald et al., 2022). This would be consistent with BMMs.

In conclusion, currently, there is no evidence that sugar transport to the fungus is regulated by nutrient supply from the fungus at the symbiotic interface, but there are multiple possible mechanisms by which transport could be linked, which require further investigation.

III. Looking forward: newideas, testable hypotheses, and future experiments

In our quest to understand what determines the transfer of carbon from plants to fungi, we have examined two broad frameworks, BMMs and Surplus C hypotheses. We determined that a critical difference between the two frameworks is whether the transfer of carbon from the plant is directly regulated by the transfer of nutrients from the fungi.

After synthesizing three bodies of literature, we found no empirical evidence of direct regulation. Nevertheless, the possibility remains open. We lack knowledge (e.g. cellular mechanisms regulating membrane transport in plants and fungi) and have hurdles to overcome in experimental work (e.g. fully measuring the carbon sink strength of mycorrhizal fungi). Current experiments cannot differentiate between frameworks, and inconsistent use of terminology (Box 1) hampers our ability to discuss concepts. We have identified some knowledge gaps and ideas for future experiments to better understand what determines the transfer of carbon from plants to mycorrhizal fungi.

1. Is carbon transferred to fungi based on their relative sink strength or nutrient delivery?

Results from many choice experiments agree with both BMMs and Surplus C frameworks, and fungal sink strength needs to be decoupled from benefit provided. In other words, experiments should include fungi that are the biggest sink but do not provide the most benefit. Biological Market Models would predict that carbon is transferred to the fungus providing more benefits independent of its sink strength, whereas Surplus C hypotheses would predict transfer based on sink strength (see Gavito *et al.*, 2019 for good methods to assess sink strengths). As discussed in the vote-count exercise above, this requires a full account of carbon transferred to mycorrhizal fungi, including respiration, which we rarely measure. However, investigating carbon fluxes or their outcomes on their own may still be inconclusive, and an increased knowledge about direct and indirect controls of carbon and nutrient transfers on the cellular level is clearly needed.

2. Reconsidering the term 'surplus-carbon'

Whether carbon is 'surplus' or not does not distinguish between frameworks, but it is an important concept when considering relative costs and benefits of exchanging resources. Unfortunately, the term 'surplus-carbon' can mean different things to different researchers. Whether carbon being transferred to fungi is in surplus of plant needs has historically been evaluated by measuring plant growth. However, a broader view of surplus-carbon recognizes all of a plant's carbon needs. These two views of surplus-carbon could be the same if growth is the lowest priority sink for plants (i.e. plants allocate carbon to growth only when all other sinks are met). If not, historical measures of surplus-carbon should more correctly be called 'surplus-carbon for growth'. Unfortunately, how plants regulate and prioritize carbon allocation to competing sinks is a major research gap in plant biology (Dietze et al., 2014; Thompson, 2024), and it remains unclear which plant carbon sinks in addition to growth we should measure before declaring carbon 'surplus'. Additionally, surplus-carbon implies carbon that a plant cannot use. Yet, carbon transferred to mycorrhizal fungi ultimately serves a plant function, to acquire nutrients. In that sense surplus-carbon may not actually be surplus (further addressed below).

3. Should mycorrhizal fungi be considered extensions of roots?

In both BMMs and Surplus C hypotheses, there is a separation between plant and fungal metabolism, but is this realistic? Metabolic integration is common in obligate symbionts, particularly endosymbionts, and some degree of metabolic integration between plants and mycorrhizal fungi is expected. Because the carbon transferred to the fungal partner is ultimately used for nutrient uptake, assimilation, and supply to the plant, it may be viewed as an extension of the processes needed to resume plant growth. Should we then consider the fungus a metabolic extension of the plant, even if only partial or temporary? We need to understand what determines the switch from plants using sugars for root growth and direct nutrient uptake to using sugars for fungal growth and indirect (through the fungus) nutrient uptake. Recent findings show that transcription factors involved in the plant phosphorus starvation response are required for mycorrhizal colonization (Shi et al., 2021), which suggests regulation depends on plant nutrient status.

4. At what spatial scale can plants regulate carbon flow to mycorrhizal fungi?

Plant carbon is allocated to different parts of the roots based on nutrient-sensing and/or carbon sink strength (Corrêa *et al.*, 2012,

2023), but the spatial resolution is unclear. At least four AM fungal taxa can co-occur in a 5-cm root piece (Jansa et al., 2003), and colonized EcM root tips can harbor multiple fungal taxa (Dietrich et al., 2022). If carbon transfer from plants to fungi is only regulated at a coarse-scale, it would be taken up by co-occurring fungi. If, however, carbon transfer responds to individual differences in carbon removal or nutrient transfer of associated fungi at the symbiotic interface, fine-scale control of transfer is possible. For AM fungi, it has been suggested that the recognition happens at the arbuscule level by the root sensing a phosphorus patch (Fitter, 2006). However, varying the spatial scale of co-occurring fungi has shown more carbon being transferred to the 'better' mutualist when fungi were spatially separated, but not when they co-occurred (Bever et al., 2009; Hopkins et al., 2023), which is inconsistent with fine-scale recognition (but see Kiers et al., 2011). For EcM fungi, Mayerhofer et al. (2021) discovered strong spatial coupling between carbon and nitrogen at the cellular scale in EcM beech roots. This could be driven by reciprocal reward mechanisms but could also result from hyphae more actively acquiring nitrogen being stronger carbon sinks. Regardless, it indicates that feedback mechanisms may operate at the scale of individual hyphae in EcM, which is considerably smaller than usually considered. The same experiment found much weaker relationships across root sections, indicating that different controls of carbon flow may exist at different scales. Overall, knowing more about the small-scale spatial distribution and physiology of both EcM and AM fungi is crucial for enhancing our understanding of resource transfer in the mycorrhizal interface. To that extent, emergent technologies capable of tracking molecules on cellular levels, such as NanoSIMS and nutrient-coated quantum dots (Whiteside et al., 2019; Mayerhofer et al., 2021) are invaluable so long as tracers accurately reflect resources being transferred (Raven, 2022).

5. Could source-sink as a null model be useful?

Like Hubbel's neutral theory of biodiversity, the suggestion that Surplus C Disposal is the simplest explanation for mycorrhizal interactions has been controversial (Prescott *et al.*, 2020; Noë, 2021; Prescott, 2022). Yet, the simplicity of using only source–sink dynamics to explain carbon transfer to fungi, as the Surplus C Disposal Hypothesis does, may have value. We second the suggestion by Bogar (2023) to consider source–sink dynamics as a null model, at least within each symbiont and extend this idea to include both AM and EcM. We are not necessarily suggesting source–sink is the correct model, just that complexity should only be added when observations deviate from this model. Analogies and metaphors can help develop new ideas, but they can also be counterproductive if they create expectations that bias our interpretation of the evidence.

6. How do Surplus C hypotheses work over evolutionary time?

Biological Market Models are cast as evolutionary models to understand the origins and maintenance of mycorrhizal mutualisms without claims on understanding physiological mechanisms, whereas Surplus C hypotheses emerged from physiological experiments with few connections to evolution. If carbon is simply directed toward fungi who remove most sugars from the apoplast regardless of fungal nutrient transfer, how would mutualism be maintained? Bogar (2023) highlighted the possibility that plants may limit carbon transfer to EcM fungi that deliver less nutrients by activating various immune responses, which, incidentally, is what happens with pathogens. However, even in the absence of this, maintenance of mutualisms may not require reciprocal rewards as plants that obtain more benefits from mycorrhizal fungi will be healthier and more fit, which ultimately will benefit mycorrhizal fungi. Modelers should implement aspects of Surplus C, including source–sink dynamics, into evolutionary models to test scenarios that affect the stability of the symbiosis.

7. How could non-nutritional benefits influence carbon transfer?

While our review has focused on nutritional benefits from mycorrhizal fungi, non-nutritional benefits (e.g. pathogen protection and increased drought tolerance) exist and can sometimes surpass increased nutrient acquisition (Delavaux et al., 2017). For example, AM fungal inoculations onto 54 agricultural fields showed that plant benefits were greatest where putative pathogen relative abundance was highest, not where phosphorus limitations were greatest (Lutz et al., 2023). Given that the underlying mechanisms for many of these are not resource based and do not require a tit-for-tat, Surplus C hypotheses may provide a more parsimonious explanation than BMMs. In fact, regulation based on nutrient exchange may be maladaptive as it could reduce carbon transfer to fungi that provide other services. It could even be advantageous for plants not to regulate carbon movement too closely as a bet-hedging strategy where potential future benefits may be derived from present investments.

IV. Synthesis of ideas

Given our understanding of the theoretical and experimental work that has been done to date, we tentatively suggest the following synthesis of ideas (Fig. 5). This synthesis includes and/or modifies concepts from BMMs and Surplus C Hypotheses that we found to be best supported by the current experimental evidence, including the following:

- Carbon transfer and nutrient transfer are indirectly linked at the organismal level, and strict reciprocity does not seem to occur.
- While carbon delivery from plants may not require nutrient delivery by fungi, nutrient delivery by fungi may depend on carbon transfer from plants.
- Exchange rates (ratio of carbon to nutrients transferred) vary depending on resource availability, symbiont resource efficiencies, and symbiont resource requirements.
- Plant growth responses to mycorrhizas result from increased nutrient uptake but are only dependent on carbon transferred to the fungus insofar as this affects nutrient uptake and subsequent transfer from the fungus.



Fig. 5 A proposed model for carbon transfer in arbuscular (AM) and ectomycorrhizal (EcM) symbioses. Under moderate nutrient stress, plants (1) generate photosynthates beyond what they can use for growth resulting in (2) an increase in the passive diffusion of sugars into the apoplast (symbiotic interface) that are (3) actively taken up by mycorrhizal fungi. (4) Fungi take up sugar and direct it toward metabolic needs including growth and acquisition of nutrients. Excess nutrients are transferred to plants and the amount of excess nutrients increases with the amount of sugar received by the fungus (provided nutrients are available). (5) The increasing flow of nutrient from the fungus allows plants to redirect photosynthates to growth. (6) With plant growth, the photosynthate supply to fungi decreases and fungal growth and acquisition of nutrients slows, and (7) nutrient flow to the plant decreases. Note that the arrows represent on-going and relative resource fluxes, rather than a stepwise process as outlined by the Surplus C Functional Equilibrium Hypothesis. Note also that moderate nutrient stress is a common context for plants, so this model would apply broadly, but not universally. Finally, this model makes explicit assumptions about fungal acquisition of nutrients which are implicit in the Surplus C Functional Equilibrium Hypothesis. First, fungi acquire nutrients beyond what they can use for growth, and those nutrients flow into sinks like the symbiotic interface, where plants take them up. Second, fungal nutrient acquisition and transfer to its host plant increases with increasing photosynthate received. The second assumption may seem counterintuitive when combined with our earlier assertion that carbon transferred from plants to fungi does not correlate with nutrients transferred from fungi to plants. Yet, because fungi depend primarily on plants for carbon compounds, we believe this is a reasonable hypothesis.

The synthesis we propose includes some caveats. Using plant growth to evaluate overall response to mycorrhizas is only useful if resources are allocated to growth only after all other metabolic needs are met and more work is needed to fully understand how oaded from https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.20145, Wiley Online Library on [0310/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

plants allocate carbon among their competing sinks. Additionally, we mostly discuss 'mycorrhizas' as one group because AM and EcM appeared similar in many ways. But, AM and EcM involve different taxa, evolutionary pathways, different structures, and the limited ability by some EcM fungi to acquire carbon by oxidizing organic matter (Lindahl & Tunlid, 2015). Future studies on these two mycorrhizas, as well as ericoid and orchid mycorrhizas, may identify distinctly different mechanisms that require different models.

V. Conclusions

Many of us begin our papers by stating that in the mycorrhizal symbiosis plants deliver carbon to fungi in exchange for nutrients delivered by fungi. Many of us have also assumed that this exchange is directly coupled, and amounts transferred are based on market economics. Markets are familiar to us, but the inner workings of mycorrhizas are not, which may explain the difficulty we have in imagining other possibilities. The idea that 'prices' do not regulate carbon transfer to fungi but instead carbon is drawn to the strongest sink is one such possibility. That exchanges are regulated through the integration of carbon and nutrient metabolisms within plant cells and possibly across plant and fungal membranes is another. That separate mechanisms may control carbon transfer and nutrient delivery puts us outside a market situation where goods are traded between individuals. Fungal parasitism is foreclosed if carbon transferred to fungi is not at the expense of plant growth. These conclusions emerged from over a year of exciting, sometimes frustrating, but always intriguing discussions, and the persistent disagreements among us highlight the work still ahead.

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Competing interests

None declared.

Author contributions

This paper is the product of a working group that was started by JK and CEP and met regularly from October 2022 to May 2024. All authors participated in discussions and edited the manuscript. RAB set agendas and moderated discussions from September 2023 onward. AC led transporter literature synthesis with help from JJ and CK. JK, AC, and RAB led history of frameworks. YL searched literature and extracted data for vote-counting and meta-analyses. YL completed vote-counting with input from AC. RAB ran meta-analyses with input from JK, AS, and YL. AS drafted plant physiology sections. RAB and AC contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Methods S1 Vote-counting methods.

Methods S2 Shade meta-analyses methods.

Table S1 Vote-counting results.

Table S2 Shade meta-analyses variables.

Table S3 Shade meta-analyses covariates.

Table S4 Shade meta-analyses results.

Table S5 Raw data for the first meta-analysis on plant response to shade.

Table S6 Raw data for the second meta-analysis on fungal response to shade.

Table S7 Raw data for the third meta-analysis on plant response to mycorrhizas.

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