



Tansley insight

Integrating plant carbon dynamics with mutualism ecology

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Summary

Plants reward microbial and animal mutualists with carbohydrates to obtain nutrients, defense, pollination, and dispersal. Under a fixed carbon budget, plants must allocate carbon to their mutualists at the expense of allocation to growth, reproduction, or storage. Such carbon trade-offs are indirectly expressed when a plant exhibits reduced growth or fecundity in the presence of its mutualist. Because carbon regulates the costs of all plant mutualisms, carbon dynamics are a common platform for integrating these costs in the face of ecological complexity and context dependence. The ecophysiology of whole-plant carbon allocation could thus elucidate the ecology and evolution of plant mutualisms. If mutualisms are costly to plants, then they must be important but frequently underestimated sinks in the terrestrial carbon cycle.

I. Introduction

Plants usually require nonplant mutualists (microbes or animals, or both). Two questions are of broad and persistent interest to mutualism ecologists. First, why do mutualisms persist given evolutionary pressure to minimize interaction costs (Ghoul *et al.*, 2014)? Second, given that the costs and benefits of species interactions are context-dependent (Chamberlain *et al.*, 2014), how do we predict the outcomes of mutualisms, and their effects on communities and ecosystems, across space and time (Maron *et al.*,

2014)? We need such predictions to manage populations and ecosystems successfully under rapid global change.

The cost to a plant of participating in a given mutualism, or set of mutualisms (Afkhami *et al.*, 2014), depends largely on fixed carbon (C). Microbes and animals provide plants with crucial chemical and locomotive benefits. Plants transform light energy into chemical energy and provide these mutualists with carbohydrate-based rewards. Variation in costs across mutualisms and contexts (Bronstein, 2001) is therefore often determined by the supply of C (i.e. from photosynthesis) and by the opportunity cost of C allocation to mutualists instead of to other sinks (e.g. growth, respiration, storage; Fig. 1a). Tracking C allocation patterns and their relationship to plant fitness could thus reveal core mechanisms that will allow us to predict how strongly plants will invest in their mutualists across ecological contexts.

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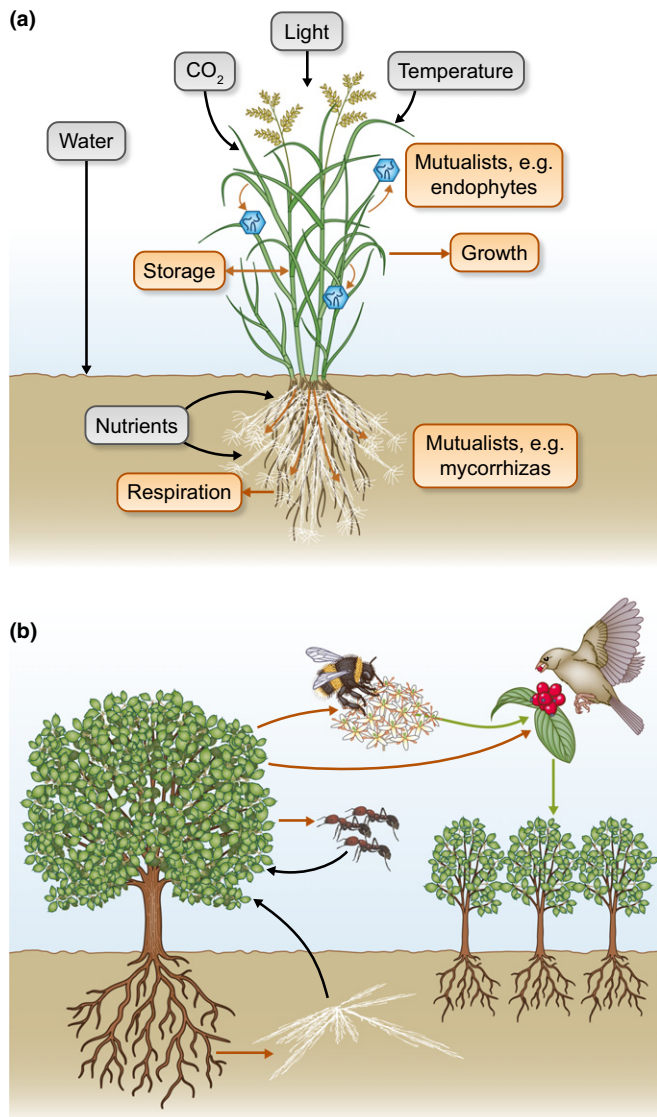


Fig. 1 Mutualisms are critical elements of plant carbon (C) cycling. The black arrows indicate factors that affect C production and orange arrows indicate C allocation by the plant. (a) A source–sink diagram for plant C that includes defense mutualists, such as leaf fungal endophytes, and nutritional mutualists, such as mycorrhizal fungi. (b) Plant mutualists provide pollination, dispersal, defense, and nutrients, but the relative C costs of these mutualists are not known. Carbon allocated to pollinators and dispersers is invested in the next generation and cannot be recovered by the individual plant. By contrast, C allocated to defensive and nutritional mutualists can feed back to the C source by increasing photosynthetic efficiency or leaf area, even producing a net C gain.

A better understanding of how plants allocate C to their mutualists, and at what cost, also holds promise for answering key questions in plant ecophysiology. For example, when does C availability limit plant growth (Palacio *et al.*, 2014)? Do plants store C actively, or is storage a passive process that occurs only when C cannot be allocated to other sinks (Dietze *et al.*, 2014)? And how does abiotic stress, in particular the increased droughts expected in some regions under climate change, affect plant C-allocation strategies (Pringle *et al.*, 2013)? Studying these questions in the context of plant mutualisms will also indicate whether and how

mutualism should be included in sink-based dynamic global vegetation models (Fatichi *et al.*, 2014).

Here, I summarize our current understanding of plant C allocation to mutualists and the implications for the ecophysiology of plant C dynamics. I then discuss promising methods that could be used to examine C dynamics explicitly in ecological studies of mutualism. I finish by discussing the potential importance of plant mutualisms to the global carbon cycle.

II. The carbon dynamics of plant mutualisms

A holistic view of plant C allocation incorporates the trade-offs among investment in different mutualist guilds and individual-level sinks (Fig. 1). Nutritional and defensive mutualists, unlike pollinators and dispersers, can offset the plant's C investment by increasing photosynthetic efficiency or area. This can establish positive feedback between the plant's C loss to the mutualist and C gain (Fig. 1b). Net benefits of nutritional and defensive mutualisms are indeed often approximated by plant growth as a proxy for fitness (Chamberlain & Holland, 2009; Johnson & Graham, 2013). Yet such mutualists could provide hidden physiological benefits even without plant growth, for example when mineral nutrients or cofactors strongly limit plant fitness (Smith & Smith, 2013). Empirical examples of such physiological subtleties come almost exclusively from plant–microbe mutualisms, perhaps because the C is exchanged for another nutrient (but see Selosse & Roy, 2009). The C that plants expend to reward their defenders, pollinators, and dispersers is no less real and potentially no less costly.

Just how costly is C to plants and, more specifically, can C availability limit plant growth and fecundity? Some physiological studies of C allocation, particularly in trees where considerable C storage is possible, argue that C availability rarely limits growth (e.g. Palacio *et al.*, 2014). Mutualism studies frequently suggest otherwise. For example, the East African myrmecophytic tree *Acacia drepanolobium* houses defensive ant colonies and provides them with C via extrafloral nectaries and scale insects. Experimentally removing the ants for 4.5 yr produced delayed increases in tree height and stem diameter relative to controls (Stanton & Palmer, 2011). In addition, experimentally reducing the density of individual ant workers on a tree increased the number of fruits in a single year (Palmer & Brody, 2013). Similar trade-offs have been observed in nutritional mutualisms (Johnson & Graham, 2013; Regus *et al.*, 2015) and, though rarely studied, are predicted for pollination and dispersal mutualisms (Southwick, 1984; Bronstein, 2001). Plants may also discriminate between mutualists on the basis of C costs. For example, Kiers *et al.* (2011) demonstrated preferential C allocation by *Medicago truncatula* plants to the mycorrhizal species that requires the least C per transferred unit of phosphorus.

The potential for plant C limitation and its effects on C allocation to mutualists can also be investigated by experimental manipulation of CO₂, light, or water. Below I consider the evidence to date from each of these manipulations.

CO₂ experiments

Plants usually increase growth upon initial exposure to elevated CO₂, which suggests that they often experience some C limitation

(Dietze *et al.*, 2014). Allocation to mutualism may also increase under elevated CO₂. For example, mycorrhizal colonization of *Asclepias syriaca* milkweed plants following caterpillar herbivory was higher under elevated CO₂ than under ambient CO₂, suggesting that carbon limits the plant's ability to acquire nutrients for leaf reconstruction (Vannette & Hunter, 2014). In the same study, however, herbivory by phloem-feeding aphids did not decrease mycorrhizal colonization, perhaps because the size of the plant's C pool can be altered by the mutualists themselves. For example, mycorrhizal fungi can increase photosynthetic rates (Johnson *et al.*, 2015), and aphid feeding can alter C allocation among sinks (Wu & Thrower, 1973). Elevated CO₂ also increases the plant's demand for mineral nutrients. Simultaneous manipulation of CO₂ and nitrogen, for example, has demonstrated that interactions among limiting resources affect plant C allocation to mutualism and its effects on ecosystems (Cheng *et al.*, 2012; Hoover *et al.*, 2012).

Light experiments

Decreased light tends to reduce plants' C allocation to their mutualists. For example, perennial woodland orchids growing in full shade must reach a larger threshold size than individuals growing in open habitats to produce bee-pollinated flowers (Jacquemyn *et al.*, 2010). Similarly, C₄ grasses sustained higher costs from associating with mycorrhizal fungi in severe shade than in full light, and percentage fungal root colonization decreased in severe shade (Johnson *et al.*, 2015). In partial shade, however, some C₄ grasses actually benefited more from the mycorrhizas than did plants in full light, and percentage mycorrhizal colonization of these plants was similar (Johnson *et al.*, 2015).

Variation in the importance of plant C limitation among systems may explain this variability. The effect of partial shade on C₄ grasses may be small because they are rarely C-limited, which indeed may facilitate their reliance on mycorrhizal fungi for nutrient acquisition (Johnson *et al.*, 2015). At the opposite extreme, woodland orchids are apparently so C-limited that flowering in full shade reduced plant size and the probability of flowering in the subsequent year (Jacquemyn *et al.*, 2010). This suggests that flowering actually requires orchids in full shade to store C between years, and that plants put additional carbohydrates toward storage in vegetative years. The potential for stored C to regulate plant mutualisms is also suggested by studies of nectaries, both floral and extrafloral (Heil, 2015). Extrafloral nectar (EFN) attracts predators, particularly ants, which defend plants against their herbivores. Shading of individual leaves can reduce EFN production on those leaves (Millán-Cañongo *et al.*, 2014), which suggests that local, newly produced C partly supplies EFN. However, plants usually produce more EFN on valuable new leaves than on older leaves (Heil, 2015), and, because new leaves are C sinks before they are sources, this suggests that additional C must be transported from elsewhere in the plant or even stored between years in deciduous species. An additional interesting and, to my knowledge, unanswered question is whether the phenology or prevalence of EFNs differs between annual and perennial plants as a result of interannual C storage in perennials.

Water experiments

Plants under water stress close stomata and expend C to regulate water potential (Dietze *et al.*, 2014). Water stress might therefore be predicted to decrease EFN secretion, but such effects depend on plant genotype in *Populus tremuloides* (quaking aspen) trees (Newman & Wagner, 2013). In particular, there was an apparent trade-off between C allocation to EFN and drought tolerance: the genotype with the highest constitutive amounts of EFN reduced its secretion most strongly in response to drought. However, similar to results from shading experiments (Millán-Cañongo *et al.*, 2014), water stress did not affect the induction of EFN in response to herbivory (Newman & Wagner, 2013). Maintaining EFN induction in the face of C limitation is potentially highly favorable, because predators defend the C source (Pringle *et al.*, 2013).

Higher relative costs of C under water stress should consistently decrease rewards for pollinators and dispersers because C allocated to these mutualists does not feed back to the source (Fig. 1b). Nectar sugar content and phloem flow to fruits can indeed decrease with water limitation (Muniz *et al.*, 2013; Morandi *et al.*, 2014). The outcomes of nutritional mutualisms, by contrast, should depend on a balance of factors. For example, symbiotic nitrogen fixation slows before photosynthesis does under water stress, which means that water stress decreases C allocation to nitrogen-fixing rhizobia (Serraj *et al.*, 1999). However, both rhizobia and mycorrhizas can increase photosynthetic rate and water-use efficiency (e.g. Birhane *et al.*, 2012), which could offset the plant C allocated to microbial mutualists, making the balance of costs and benefits more favorable.

As outlined earlier, the outcomes of plant mutualisms under a given set of abiotic conditions are contingent on the costs of C allocation and its effects on plant performance. For example, like water stress, soils that are rich in mineral nutrients increase the relative cost of C to the plant. Yet, unlike water stress, nutrient-rich soils should consistently weaken nutritional mutualisms because transferred nutrients accrue low benefits per unit C cost (Werner & Kiers, 2015). A more explicit focus on C allocation to mutualism could thus help to elucidate the causes and consequences of context dependence.

III. Tracking carbon allocation to mutualism

One promising approach for elucidating C budgets is to measure nonstructural carbohydrates (NSCs) and compare them among plants, across tissues, and through time (Hoch, 2015). Importantly, such measures are relative: for example, high NSC reserves may mean that the plant is healthy, or that it is severely sink-limited as a result of some other environmental stress. It is still unclear whether plants store NSCs actively (as opposed to only passively when C cannot be allocated to other sinks) and, if so, when and why, as well as how far reserves can be depleted before plants experience greater mortality risk (Dietze *et al.*, 2014). In addition to supplying carbon for metabolism, NSCs can play a critical role in physiological processes such as the maintenance of hydraulic function (O'Brien *et al.*, 2014). A better understanding of C dynamics, including simplified experiments using seedlings, could ultimately improve

our ability to estimate fitness for plants such as adult trees that are less amenable to the spatiotemporal scales of ecological experiments.

The C that plants allocate to mutualists must come from the pool of NSCs because structural C is tied up in cellulose and other insoluble carbohydrates. Trees may use stored NSCs to offset the costs of large fruit crops, and there is active debate about whether stored NSCs are involved in mast-fruiting (Hoch, 2015). To my knowledge, this has not been investigated for species that rely on animals for seed dispersal. However, syrup production records from wind-dispersed sugar maples (*Acer saccharum*) suggest that these trees mast when NSCs are high and that masting uses and depletes NSC stores (Rapp & Crone, 2015).

We can also measure NSCs to clarify mutualism function. In a study of a defensive mutualism along a precipitation gradient, NSC concentrations were used to determine where along the gradient trees were most water-stressed and would benefit most from ant defense (Pringle *et al.*, 2013). In another study, Brouwer *et al.* (2015) reported that an allelopathic invasive plant causes lower NSC concentrations in rhizomes of native plants by disrupting the mutualism between native plants and arbuscular mycorrhizas. This observation is consistent with mycorrhizal fungi playing an important role in water relations in this system (Hale *et al.*, 2011). By contrast, if the primary role of the mycorrhizas were phosphorus transfer, disrupting the mutualism should have led in the short-term to C sink limitation and NSC accumulation.

Nonstructural carbohydrate allocation can also be traced, in both the short and long terms, using C isotopes. ^{13}C pulse labeling can be used to track fresh assimilates into older NSC pools (Streit *et al.*, 2013) and mutualist rewards. For example, this approach has been used to identify differential C flux to arbuscular mycorrhizal fungi, as well as to previously unknown bacterial root symbionts (Vandenkoornhuyse *et al.*, 2007). Recent evidence tracking ^{14}C radiocarbon suggests that trees have distinct fast- and slow-cycling NSC pools (Richardson *et al.*, 2015), and that slow-cycling pools, accumulated over decades, can be used to respond to severe disturbances (e.g. Carbone *et al.*, 2013). It is not known whether slow-cycling NSCs can be allocated to plant mutualists.

A persistent challenge for understanding NSC dynamics has been posed by limitations in methods for studying phloem transport, particularly under field conditions. New methods are emerging, however, from measuring changes in bark thickness to estimate phloem flow (Mencuccini *et al.*, 2013), to studying the activity of phloem-loading proteins (Chen *et al.*, 2012). Using combinations of these approaches to study mutualisms will produce a much better picture of how plants regulate their C allocation to mutualists in the context of other, more studied C sinks.

IV. Mutualisms and the global carbon cycle

The manner in which plant mutualisms exert global effects will depend both on how much C is allocated to mutualists and on the mutualists' functional traits. Recent efforts to move toward sink-based vegetation modeling have included C export to mycorrhizal fungi (Faticchi *et al.*, 2014), a first step toward considering plant mutualisms as important global sinks. Increased C allocated to

ectomycorrhizas but not arbuscular mycorrhizas appears to increase soil C sequestration because only ectomycorrhizas typically outcompete free-living saprotrophic microbes for N (Cheng *et al.*, 2012; Averill *et al.*, 2014). This example also highlights the importance of scale: the magnitude of a mutualist's effect on plant fitness is not necessarily aligned with how strongly the mutualism affects the global carbon cycle. Defining the temporal scale of interest is also important. We will not know that it is 'irrelevant to describe the pollination ecology of a particular species' to estimate a forest's carbon budget (Schimel & Keller, 2015) until we know how much C is actually allocated to pollinators and at what cost to growth, but it also matters whether we are interested only in the carbon budget today or that of the same forest in 100 yr.

Mutualisms may be particularly important to the global carbon cycle under predicted increases in extreme climate events. Nutritional and defensive mutualisms could decrease the risk of plant mortality under drought, attenuating the potentially dramatic effects of droughts on the global carbon cycle (Frank *et al.*, 2015). Amazonian trees appear to prioritize C allocation to above-ground growth after drought at the expense of respiration and below-ground growth (Doughty *et al.*, 2015), which could decrease C allocated to mutualists. It will be important to know to what extent such effects increase tree mortality and reduce new tree recruitment.

V. Conclusions

If plants were rarely C-limited, then mutualisms would rarely be costly. Evidence to date actually suggests widespread C trade-offs between mutualists and plant growth and reproduction, indicating that mutualisms are important carbon sinks. An explicit focus on the flow of energy through mutualisms would elucidate the repercussions of different C allocation strategies, both for individual plant fitness and for carbon cycles on larger scales. Using C as a common currency to track complex plant interactions is also a first step toward a more complete approach that considers the trade-offs and functional traits of plant mutualists. Mutualisms came late to ecological theory, but the time is ripe to consider their importance to global vegetation models.

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