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Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses?

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ABSTRACT

Rhizobial and arbuscular mycorrhizal (AM) symbioses each may consume 4–16% of recently photosynthetically-fixed carbon to maintain their growth, activity and reserves. Rhizobia and AM fungi improve plant photosynthesis through N and P acquisition, but increased nutrient uptake by these symbionts does not fully explain observed increases in the rate of photosynthesis of symbiotic plants. In this paper, we test the hypothesis that carbon sink strength of rhizobial and AM symbioses stimulates the rates of photosynthesis. Nutrient-independent effects of rhizobial and AM symbioses result in direct compensation of C costs at the source. We calculated the response ratios of photosynthesis and nutrient mass fraction in the leaves of legumes inoculated with rhizobial and/or AM fungi relative to non-inoculated plants in a number of published studies. On average, photosynthetic rates were significantly increased by 28 and 14% due to rhizobial and AM symbioses, respectively, and 51% due to dual symbiosis. The leaf P mass fraction was increased significantly by 13% due to rhizobial symbioses. Although the increases were not significant, AM symbioses increased leaf P mass fraction by 6% and dual symbioses by 41%. The leaf N mass fraction was not significantly affected by any of the rhizobial, AM and dual symbioses. The rate of photosynthesis increased substantially more than the C costs of the rhizobial and AM symbioses. The inoculation of legumes with rhizobia and/or AM fungi, which resulted in sink stimulation of photosynthesis, improved the photosynthetic nutrient use efficiency and the proportion of seed yield in relation to the total plant biomass (harvest index). Sink stimulation represent an adaptation mechanism that allows legumes to take advantage of nutrient supply from their microsymbionts without compromising the total amount of photosynthates available for plant growth.

1. Introduction

Legumes associated with rhizobia and arbuscular mycorrhizal (AM) fungi show improved performance and higher yields than non-symbiotic plants. These positive effects of rhizobial and AM symbioses have been attributed to an improved nutritional state (due to N supplied by rhizobia and P by AM fungi), which in turn leads to increased photosynthetic rates and improved plant growth. Simultaneously, there is a cost to the legume of rhizobial and AM symbioses, as each may consume as much as 4–16% of recently fixed photosynthetic carbon to maintain their activity (Table 1). The photosynthate (C) derived from photosynthesis to maintain the performance of these symbioses is often referred to as the "cost", and the nutrients obtained through the symbioses are often referred to as the "benefit" of the symbiont (Koide and Elliot, 1989). The N and P acquired are the benefits from rhizobia and AM fungi, respectively, and the C costs are expressed in terms of g C g^{-1} N and g C g^{-1} P. There is evidence that AM fungi also play a role in the uptake of nitrate and ammonium (e.g. Olsson et al., 2005; Smith and Read, 2008) which are assimilated and transported within the mycelium as arginine, but compared with ectomycorrhizas, rates of N uptake by AM hyphae are too small to contribute substantially to plant N nutrition (Smith and Read, 2008).

The C costs of N acquisition by N_2-fixation are compared with N acquisition by NO_3^- uptake, based on several methods in Table 2. The C costs of N_2-fixation are almost exclusively incurred in the biochemical reactions of N_2-fixation (Witty et al., 1983; Ryle et al., 1984; Voisin et al., 2003). On a theoretical basis, the C costs of N_2-fixation should range between 3.3 and 6.6 g C g^{-1} N, depending on the legume–rhizobia combination, whereas NO_3^- reduction should not exceed 2.5 g C g^{-1} N (Atkins, 1984; Minchin and Witty, 2005).

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Table 1
Carbon sink strength of symbioses and its effect on the rate of photosynthesis of legumes.

Plant species	AM fungi or rhizobia species	C allocation to symbiont (%)	Increase in net photosynthesis (%)	Method	Reference
Rhizobia					
Glycine max	*Bradyrhizobium* spp.	11	ND	Growth/respiration analysis	Ryle et al. (1979a)
G. max	*Bradyrhizobium japonicum*	14	28	Growth/respiration analysis	Finke et al. (1982)
G. max	*B. japonicum*	9	24**	$^{14}CO_2$ allocation	Harris et al. (1985)
G. max	*B. japonicum*	9	16**	$^{14}CO_2$ allocation	Harris et al. (1985)
Vicia faba	*Rhizobium leguminosarum*	6	13**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*R. leguminosarum*	ND	8**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*R. leguminosarum*	4	5*	$^{14}CO_2$ allocation	Paul and Kucey (1981)
Vigna unguiculata	*Bradyrhizobium* spp.	13	ND	Growth/respiration analysis	Ryle et al. (1979a)
Trifolium repens	*Rhizobium* spp.	13	ND	Growth/respiration analysis	Ryle et al. (1979a)
AM fungi					
G. max	*Glomus fasciculatum*	16	23**	$^{14}CO_2$ allocation	Harris et al. (1985)
G. max	*G. fasciculatum*	7	6^{ns}	$^{14}CO_2$ allocation	Harris et al. (1985)
V. faba	*Glomus mosseae*	4	8**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*G. mosseae*	ND	3**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*G. mosseae*	11	22	$^{14}CO_2$ allocation	Pang and Paul (1980)
V. faba	*G. mosseae*	7	9**	$^{14}CO_2$ allocation	Paul and Kucey (1981)
Rhizobia + AM fungi					
G. max	*B. japonicum + G. fasciculatum*	28	12**	$^{14}CO_2$ allocation	Harris et al. (1985)
G. max	*B. japonicum + G. fasciculatum*	19	7**	$^{14}CO_2$ allocation	Harris et al. (1985)
V. faba	*R. leguminosarum + G. mosseae*	16	17**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*R. leguminosarum + G. mosseae*	ND	36**	$^{14}CO_2$ allocation	Kucey and Paul (1982b)
V. faba	*R. leguminosarum + G. mosseae*	16	17**	$^{14}CO_2$ allocation	Paul and Kucey (1981)

In all studies, the rates of photosynthesis were measured as C or CO_2 uptake per g leaf dry weight per time. ND means that values were not determined. The difference in photosynthesis was calculated as: Difference (%) = 100 × [(Photosynthesis in Symbiotic plants/Photosynthesis in Fertilized plants)$^{-1}$].
Statistics as given by the authors: **significant at $P < 0.05$, *significant at $P < 0.10$, ns = differences are not significant; no symbols means that no statistical test was presented.
Data of Harris et al. (1985) were obtained at 6 and 9 weeks, respectively. Data of Kucey and Paul (1982b) was obtained at 5 and 6 weeks, respectively.

Except for pea (*Pisum sativum* L.) (Minchin and Pate, 1973), the costs of N acquisition through rhizobia are always higher than by NO_3^- uptake (Table 2). The differences in C costs may be small and not always statistically significant, but when integrated over the whole growth cycle the costs may be substantial.

Literature on the C costs of P uptake via AM symbioses is less abundant. By analyzing the radio-labelled $^{14}CO_2$ allocation patterns, Harris et al. (1985) determined that mycorrhizal roots spent 199 g C g^{-1} P whereas non-mycorrhizal roots receiving N fertilizer or inoculated with rhizobia spent 129 and 127 g C g^{-1} P, respectively. The carbon costs of P uptake by roots were only 130 and due to mycorrhizal hyphae was twice as large (267 g C g^{-1} P) (Harris et al., 1985). Smith and Read (2008) argue that C costs based on length are less for mycorrhizal hyphae because they are much thinner than roots and can exploit larger soil volumes for the same amount of C. The C costs of AM symbioses are mainly determined by the growth and maintenance of both intraradical structures (vesicles, arbuscules, spores, hyphae) and extraradical mycelium (plus spores) (e.g. Peng et al., 1993; Johnson et al., 2002), as effective AM symbioses require an extensive hyphal network. There have been few measurements of the C costs of mycorrhizal fungi (e.g. Bryla and Eissenstat, 2005) but most relate the C costs directly to a proportion of the rates of photosynthesis (Table 1). The fraction of fungal tissue in the mycorrhizal root biomass increases from 2 to 13% in soybean (*Glycine max* (L.) Merr.) (Bethlenfalvay et al., 1982a, 1982b; Harris et al., 1985; Pacovsky and Fuller, 1988), 5 to 14% in *Centrosema pubescens* Benth. (Hepper, 1977), 6 to 7% in subterranean clover (*Trifolium subterraneum* L.) (Olsson and Johansen, 2000) and 0.5 to 5% in faba beans (*Vicia faba* L.) (Kucey and Paul, 1982a) depending on the mycorrhizal fungal species, plant development, soil P supply and growth conditions. Theoretical costs of fungal growth could be calculated considering a quantitative assessment of fungal composition – proportions of carbohydrates, lipids, proteins, nucleic acids and mineral nutrients – multiplied by the glucose requirements for their synthesis and maintenance (cf. Penning de Vries et al., 1974). However, there is much uncertainty about the exact composition of AM fungi, although Bago et al. (2003) and others have indicated that C metabolism in mycorrhizal hyphae is driven by constant synthesis and degradation of lipids. In addition, the energy demand associated with lipid metabolism

Table 2
Carbon costs for N acquisition in nodulated and N-fertilized legumes.

Plant species	*Rhizobium* strain	N_2-fixation (g C g^{-1} N)	NO_3^- reduction (g C g^{-1} N)	Method	Reference
Cajanus cajan	IPH 159	5.0–14.3	ND	C:N balance/respiration analysis	Rao et al. (1984)
Glycine max	CB 1809 and CB 756	6.3	ND	C:N balance/respiration analysis	Ryle et al. (1979b)
G. max	Not identified	5.8	ND	CO_2 vs. ARA/H_2 regression	Patterson and LaRue (1983)
G. max	USDA G3	2.5–7.6	ND	$^{14}CO_2$ vs. $^{15}N_2$ allocation	Warembourg (1983)
G. max	USDA 3I1B71	7.1	4.3	C:N balance/respiration analysis	Finke et al. (1982)
Lupinus albus	WU 425	10.2	8.1	C:N balance/respiration analysis	Pate et al. (1979)
Pisum sativum	V 200	5.9	6.2	C:N balance/respiration analysis	Minchin and Pate (1973)
Trifolium repens	CB1809 and CB 756	6.6	ND	C:N balance/respiration analysis	Ryle et al. (1979b)
Vigna unguiculata	CB 756	12.3	3.7	C:N balance/respiration analysis	Minchin et al. (1980)
V. unguiculata	CB 1809 and CB 756	6.8	ND	C:N balance/respiration analysis	Ryle et al. (1979b)

Values represent the mean of observations in each study. ND means that C costs of NO_3^- reduction were not determined in the study.

Table 3
Biochemical composition of several arbuscular mycorrhizal fungi in different plant species.

Plant species	AMF species	Compound	Estimates (mg g^{-1} fungal dry weight)	Reference
Allium porrum	*Glomus* spp.	Lipids (vesicles)	582.0	Jabaji-Hare et al. (1984)
Cucumis sativus	*Glomus intraradices*	Lipids (spores)	200.0	Olsson and Johansen (2000)
Glycine max	*Glomus fasciculatum*	Lipids (hyphae)	28.5	Pacovsky and Fuller (1988)
C. sativus	*G. intraradices*	Lipids (hyphae)	19.2	Olsson and Johansen (2000)
G. max	*G. fasciculatum*	Chitin (cell wall)	88.5	Bethlenfalvay et al. (1982a)
Trifolium pratense	*G. intraradices*	Chitin (cell wall)	82.9	Frey et al. (1994)
T. pratense	*Gigaspora gigantea*	Protein	63.0	Wright et al. (1996)
Zea mays + *Sorghum sudanense*	*Gigaspora rosea*	Protein	60.0	Wright et al. (1996)
Z. mays + *S. sudanense*	*G. intraradices*	Protein	29.0	Wright et al. (1996)
Z. mays + *S. sudanense*	*G. intraradices*	Protein	21.0	Wright et al. (1996)
Z. mays + *S. sudanense*	*G. intraradices*	Protein	17.0	Wright et al. (1996)
Z. mays + *S. sudanense*	*Glomus etunicatum*	Protein	12.0	Wright et al. (1996)
Not given	*Gigaspora margarita*	Trehalose + Glucose	16.6	Bécard et al. (1991)
Not given	*G. intraradices*	Trehalose + Glucose	16.3	Bécard et al. (1991)
Not given	*G. etunicatum*	Trehalose + Glucose	0.6	Bécard et al. (1991)
Lotus corniculatus	*Glomus mosseae*	Nucleic acid (hyphae)	0.2	Bütehorn et al. (1999)
Allium cepa	*G. mosseae*	Phosphate (40% is Poly-P)	3.8	Callow et al. (1978)
A. cepa	*G. margarita*	Phosphate (10% is Poly-P)	2.5	Solaiman et al. (1999)

In the study of Olsson and Johansen (2000), the proportion of dry weight was 90% in spores and 10% in hyphae, which included vesicles.

would increase the C costs of AM symbioses. Few studies have reported quantitative assessments (Table 3), but even if we take the largest estimates, the sum of the components is not more than half of the total dry weight. Table 4 gives hypothetical fungal compositions of 5–30% of carbohydrates, 20–60% of lipids and 10–50% of N compounds. These estimates suggest that the C costs of growth and fungal respiration vary from 400 to 1500 mg C g^{-1} fungal tissue (Table 4).

Furthermore, there is evidence that C costs of both rhizobia and AM fungi are additive (e.g. Harris et al., 1985). Dual symbioses are likely to have an additive effect on the C costs if AM symbioses alleviate deficiency of P and micronutrients, and indirectly stimulate the rate of N_2-fixation, or if the enhanced N status of N_2-fixing legumes creates more demand for P (Smith and Read, 2008).

If the C invested in the symbioses is not, or insufficiently, compensated by enhanced nutrient acquisition, growth of symbiotic plants will be less than that of non-symbiotic plants. However, there is evidence for a nutrient-independent effect of the symbioses, in which the C costs are compensated directly at the source by increased photosynthetic rates (Table 1). In fact, photosynthesis may increase due to the C sink strength of the symbioses (Pang and Paul, 1980; Harris et al., 1985; Wright et al., 1998a, 1998b; Mortimer et al., 2008), and as a consequence, more C is fixed per time and per unit of nutrient, resulting in higher photosynthetic nutrient use efficiency (Brown and Bethlenfalvay, 1988; Fay et al., 1996).

In this review, we consider the potential effects of rhizobial and AM symbioses on the rates of photosynthesis, using the following questions to guide our literature analysis:

a) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and AM symbioses?
b) What is the evidence of sink stimulation under symbiotic conditions?
c) Is the magnitude of sink stimulation by rhizobia and AM symbioses similar?
d) Does sink stimulation of photosynthesis by symbioses increase yield?
e) Is sink stimulation by rhizobia and AM symbioses quantifiable, or does it remain a theoretical concept?

2. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and AM symbioses?

2.1. Limiting processes of photosynthesis

Plant photosynthesis can be expressed on a leaf area/mass basis or on a whole plant basis, and it is important to realize that different measuring approaches may lead to different conclusions. The general assumption is that rhizobial and AM symbioses affect the whole plant photosynthesis because they improve plant nutrition and growth (by increasing total leaf area), but there is also evidence that rates of photosynthesis per unit of leaf area may be increased.

We describe some of the processes that regulate photosynthesis when a leaf is affected by the metabolism of the whole plant in Fig. 1. Photosynthesis produces assimilates which are loaded into the phloem to be partitioned over the different tissues acting as

Table 4
Likely fraction of compounds of dry weight of arbuscular mycorrhizal mycelium and the theoretical C costs for biosynthesis of fungal tissue for a mature symbiosis.

Compound	g compound [g fungal biomass]$^{-1}$ (A)	g compound [g glucose]$^{-1}$ (B)	mg C required [g fungal tissue]$^{-1}$ (C)	g CO_2 released [g glucose]$^{-1}$ (D)	mg C released [g fungal tissue]$^{-1}$ (E)
Carbohydrates	0.05–0.30	0.87	23.0–137.9	0.057	0.9–5.4
Lipids	0.20–0.60	0.36	222.2–666.7	0.471	71.4–214.1
N compounds	0.10–0.50	0.48	83.3–416.7	0.249	14.1–70.7
Nucleic Acids	0.01–0.05	0.57	7.0–35.1	0.043	0.2–1.0
Mineral uptake	0.05–0.10	20.00	1.0–2.0	–	–
Total C costs		–	336.5–1258.4		86.6–291.2

(A) are hypothetical values.
(B) and (D) are values extracted from Penning de Vries et al. (1974), assuming that AMF takes up NO_3^- for its own growth.
(C) = (A)/(B) × (12/30) × 1000, where 12/30 converts glucose into C.
(E) = (A) × (D)/(B) × (12/44) × 1000, where 12/44 converts CO_2 into C.

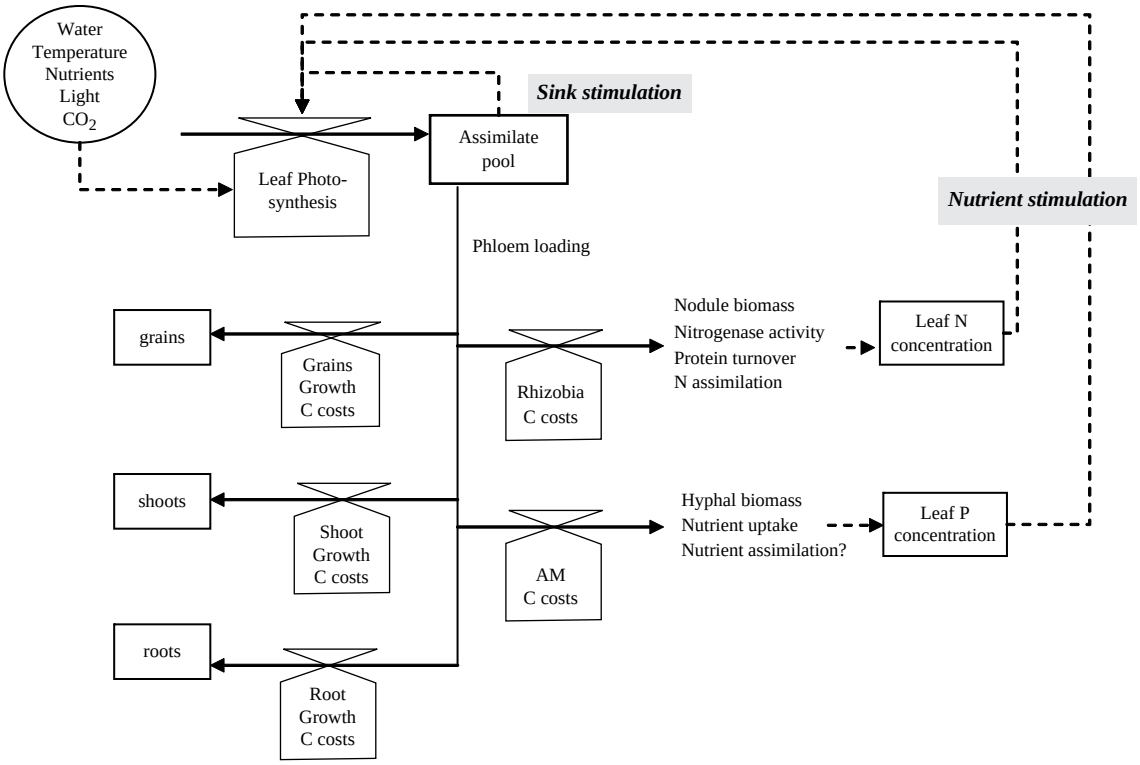

Fig. 1. Conceptual model depicting the effects of rhizobial and AM symbioses on the photosynthesis of a leaf being affected by the metabolism of the whole plant. Symbols follow the Forrester notation (Forrester, 1961). The effects of nutrient and sink stimulation are highlighted.

sinks, and respiratory processes (e.g. Yin and van Laar, 2005). Here, the C sink strength of the symbioses is a fraction of photosynthates loaded into the phloem to support either rhizobial or AM symbioses. Nutrient fertilization will increase the growth rates of shoots and increase the plant size. Leaf photosynthesis will remain at its steady state, but the overall C assimilation will increase on a whole plant basis, because of an increase in total leaf area (e.g. Lambers et al., 1998). However, if plants are dependent on rhizobial and AM symbioses, they will have additional C costs, which will increase the rates of phloem loading (Fig. 1). The C costs of rhizobia symbiosis will increase according to the nitrogenase activity, nodule biomass, protein turnover and N assimilation, and the C costs of AM symbiosis will increase according to the formation of fungal biomass, nutrient uptake and possibly by nutrient assimilation (see also Section 2.4). On the one hand, rhizobia and AM symbioses increase the nutrient mass fraction in leaves (namely N and P) and therefore may stimulate the rate of photosynthesis – nutrient stimulation (Section 2.2). On the other hand, the C costs of rhizobial and AM symbioses increase the rate of phloem loading, and therefore, stimulate the rate of photosynthesis – sink stimulation (Section 2.3).

Our current understanding is that leaf photosynthesis is limited by three biochemical processes: rubisco (ribulose-1,5-biphosphate carboxylase/oxygenase, E.C. 4.1.1.39) activity, electron transport rates and consequent ribulose-1,5-biphosphate regeneration (Farquhar et al., 1980), and triose-P utilization (Sharkey, 1985). Water availability, temperature and nutrients, particularly N, P, and enzyme components and co-factors (i.e. Mg, Fe, Cu, Mn) are important for the proper functioning of these photosynthetic processes (Lambers et al., 1998; Cakmak and Engels, 1999). Additionally, rubisco activity is limited by atmospheric CO_2 concentration, the electron transport rate is limited by light availability, and the triose-P utilization is limited by the plant C sink strength (Farquhar et al., 1980; Sharkey, 1985; von Caemmerer, 2000). Therefore, we assume that rhizobia and AM symbioses affect photosynthesis by removing the limitation of rubisco activity and electron transport rates through increases in leaf N and P mass fraction. Additionally, rhizobia and AM symbioses and its related C costs increase photosynthesis by removing the triose-P utilization limitation of photosynthesis.

2.2. Role of N and P acquisition

In rhizobial symbioses, the bacterial enzymatic complex nitrogenase (E.C. 1.18.6.1) breaks the highly-stable triple bond of N_2 and reduces it to NH_3. If a successful symbiosis is established, biological N_2-fixation can supply the majority of the N required by legumes (Zapata et al., 1987; Hungria et al., 2005). Nitrogen is essential for the synthesis of rubisco and for the synthesis of light-harvesting chlorophyll (Evans, 1989; Hikosaka and Terashima, 1995). As N_2-fixation enhances leaf N mass fraction, it should stimulate the rate of leaf photosynthesis by increasing rubisco activity and electron transport rates (e.g. Harley et al., 1992).

The relationship between N mass fraction in the leaves and the rates of photosynthesis of C_3 plants is not consistently linear. In fact, the gain in photosynthesis decreases gradually with increases in rubisco content (Mächler et al., 1988; Hikosaka and Terashima, 1995; Nelson and Cox, 2004), and N partitioning in the leaves changes according to the light environment (Hikosaka and Terashima, 1995), and/or photosynthate partitioning (Ono et al., 2001). Photosynthesis may not increase above a threshold of leaf N sufficiency ($\sim$2% on dry weight basis) (e.g. Yin and van Laar, 2005).

AM symbioses improve P acquisition by plants because the extraradical mycelium grows beyond the nutrient depletion zone of the root system (Khaliq and Sanders, 2000; Smith et al., 2003; Grimoldi et al., 2005; Cardoso and Kuyper, 2006). Some plants are

dependent on the P supply from the AM symbioses to grow well (Smith and Read, 2008). In photosynthesis, P is used for energy supply (ATP and NADPH), participates in the regeneration of the CO_2 acceptor ribulose biphosphate (RUBP), and regulates the ratio of starch:sucrose biosynthesis (Cakmak and Engels, 1999; de Groot et al., 2003; Rychter and Rao, 2005). But the effect of P acquisition on photosynthesis has been established only when P supply was strongly deficient (Sawada et al., 1992; Fay et al., 1996; Black et al., 2000).

Both P addition and AMF colonization increase leaf area per unit of plant biomass and thus also plant C assimilation on a whole plant basis (Lambers et al., 1998; Jia and Gray, 2004; Grimoldi et al., 2005). When plants are grown under conditions of P sufficiency or mild deficiency, leaf photosynthesis is not limited by ATP availability or rubisco activation. Then, the increase of sink organs stimulates the rate of triose-phosphate export, which recycles orthophosphate (P_i) back into the chloroplasts and triggers the enzymes that regulate photosynthesis (Fig. 2) (Flügge, 1995; Pieters et al., 2001; Rychter and Rao, 2005). Under extreme P limitation, rates of photosynthesis are reduced due to limitation in activation of the Calvin Cycle (lack of ATP and/or substrate). Low Calvin cycle activation results in low carbohydrate production in the leaves (such as of tomato, *Lycopersicon esculentum* Mill.) (de Groot et al., 2001). de Groot et al. (2003) subsequently demonstrated that P deprivation limits the carboxylation capacity, whereas N deprivation limits the rate of light harvesting and electron transport activity. Although rates of photosynthesis decrease under N and P limitation, plants may adapt to nutrient stress by maintaining a proportional relationship between photosystem II and photosystem I (de Groot et al., 2003).

2.3. Carbon sink strength regulation

Stimulation of photosynthesis has classically been attributed to the increase in nutrient supply, such as N and P and other nutrients (Lambers et al., 1998), although it has often been suggested that there is a limit to this increase (Yin and van Laar, 2005). A plant may achieve its potential rate of photosynthesis when the environmental conditions are optimal and the limitation relies on intrinsic physiological factors, such as the carboxylation rates limited by

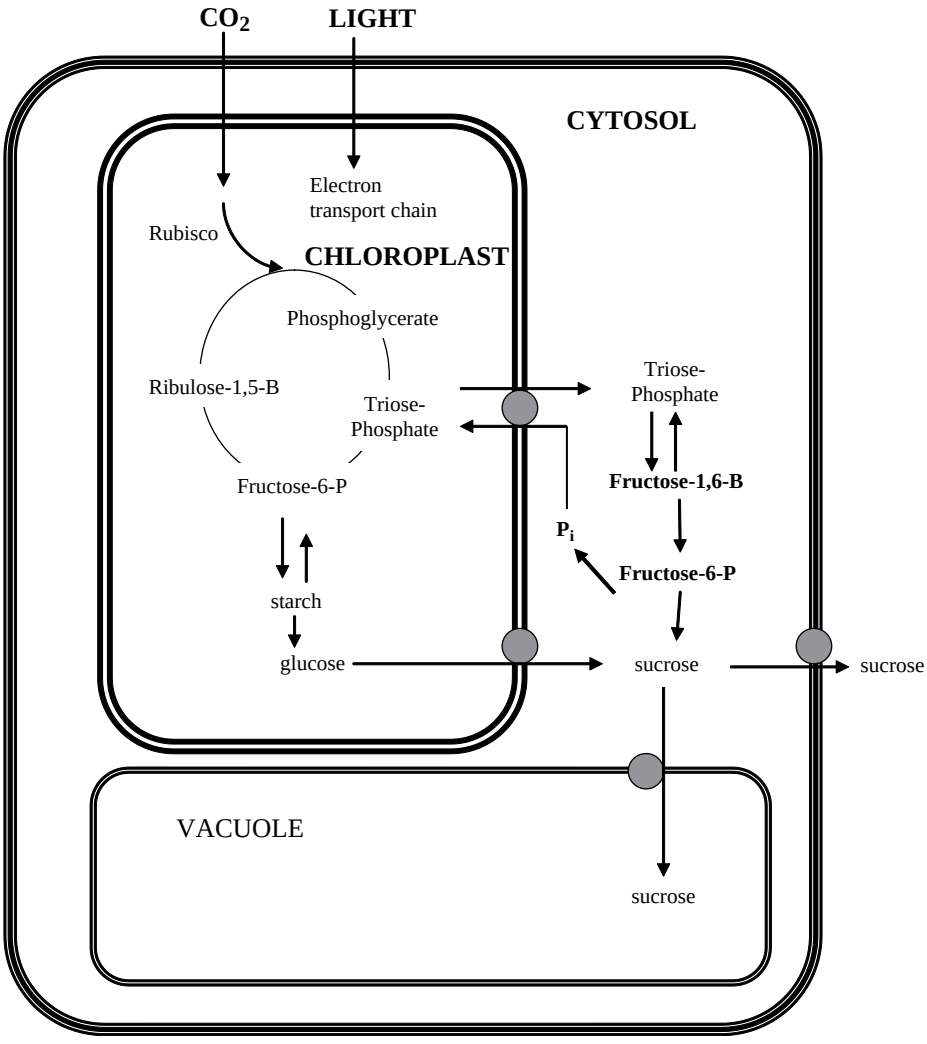

Fig. 2. Mechanisms of sucrose and triose-phosphate export from the chloroplast to the cytosol (after Flügge, 1995). The activation of rubisco leads to fixation of CO_2 to ribulose-1,5-biphosphate, which is split into two molecules of triose phosphate. While molecules of ribulose are regenerated in further steps of the Calvin Cycle, triose phosphates can be converted either into starch (in the chloroplast) or sucrose (in the cytosol). The synthesis of sucrose allows loading the phloem with photosynthates that are distributed over the sink organs; synthesis of starch occurs within the chloroplasts as a temporary strategy of energy reserve. With an increase in the sink strength, the exchange rates of triose for P_i increases and stimulates the rate of photosynthesis. Starch stored during the day in the chloroplasts can be hydrolyzed during the night but the process lags behind and cannot stimulate the actual photosynthesis.

rubisco activity and by electron transport (Farquhar et al., 1980; Yin et al., 2004; Yin and van Laar, 2005). However, the qualifier "potential" is at best conceptually imprecise and actually confusing because the upper limit is obtained from averaging observed actual maximum rates.

Once the potential rate of photosynthesis in a given situation is achieved, the rates of photosynthesis are assumed to become steady over time. The increase in photosynthates over the season is then attributed solely to increased leaf area (Yin and van Laar, 2005). Contrary to this steady-state assumption, it has been demonstrated that specific rates of photosynthesis are down-regulated during periods of low sink activity, for example, after girdling, defruiting and sink removal, because of both carbohydrate accumulation in the leaf and end-product inhibition feedback in the Calvin Cycle (Lawn and Brun, 1974; Mondal et al., 1978; Azcón-Bieto, 1983; Goldschmidt and Huber, 1992; Iglesias et al., 2002; Rychter and Rao, 2005; McCormick et al., 2006; Dingkuhn et al., 2007). Alternatively, an increased C demand stimulates photosynthetic activity, for example during the onset of flowering because reproductive organs are being formed (e.g. Lawn and Brun, 1974). The reason is that the strength of the new C sink speeds up the utilization of triose phosphate for sucrose synthesis and the export towards the phloem, increasing the P_i recycling rate when releasing P_i back to the chloroplast (Paul and Foyer, 2001) and activating the regeneration of RUBP in the Calvin Cycle (Fig. 2). Furthermore, photosynthesis is stimulated by increased triose export because the enhanced P_i availability increases the activity of the electron transport chain for the photophosphorylation of ATP and reductants, and prevents over-reduction of photosystem I (Bukhov, 2004). An increased ATP:ADP ratio enhances the activation of the rubisco provided that there is a high C demand from the sinks (Paul and Foyer, 2001).

2.4. What is the evidence for sink stimulation under symbiotic conditions?

One way to determine quantitatively sink stimulation of photosynthesis in plants that have been colonized by rhizobia and AM fungi is to compare the changes in photosynthesis and nutrient acquisition of symbiotic plants with those from non-symbiotic plants. It is possible to assess the size of such change by calculating a response ratio, a dimensionless ratio between the values of a parameter of the experimental treatment including symbiosis and the control treatment without symbiosis (Gurevitch and Hedges, 2001). Sink stimulation would be supported when the response ratio of photosynthesis is higher than the response ratio of nutrient acquisition by symbioses. To test this hypothesis, we gathered data on any study which reported both photosynthesis and leaf nutrient mass fractions as affected by rhizobial and/or AM symbioses, and calculated the response ratios (Tables 5–7). When interpreting the output of this meta-analysis, one should regard the response ratio significantly positive if the lower limit of the 95% confidence intervals (CI) is larger than 1, and negative if the upper limit of the 95% CI is smaller than 1. If the lower confidence interval is lower than 1 and the upper confidence interval higher than 1, the response ratio is not significantly different from 1. There are significant differences between the response ratios of photosynthesis and nutrient acquisition when the values of the confidence intervals of the two different response ratios do not overlap.

Tables 5 and 6 give the response ratios of rhizobial and AM legume plants, respectively. Brown and Bethlenfalvay (1987) demonstrated that neither rhizobia nor AM fungi caused an increase in the nutrient mass fractions in the leaves of soybean, but they increased the rate of photosynthesis by 5 and 17%, respectively. The differences between the response ratios of photosynthesis and nutrient mass fractions in the leaves were significant ($P < 0.05$; Tables 5 and 6). In a comparable study, Brown and Bethlenfalvay (1988) demonstrated that the C sink strength of rhizobia and AMF led to an increased photosynthetic nutrient use efficiency. We hypothesize that the C sink strength of the symbioses led to a higher rate of triose-P export, a higher rate of P_i recycling, and, as a consequence, to a higher activation state of the Calvin cycle, which implies a higher rate of CO_2 fixation in the leaves. As the entry of CO_2 through stomata was larger, there was a lower nutrient requirement for the formation of photosynthetic proteins and reductants (e.g. rubisco and ATP). Therefore, increased photosynthetic nutrient use efficiency by a symbiotic legume is an expression of sink stimulation of photosynthesis.

Analysis of the data from the study by Harris et al. (1985) demonstrated (although with low statistical significance) that a soybean symbiosis with rhizobia (Table 5) and with a combination of rhizobia and AM fungi (Table 7) resulted in higher response ratios of photosynthesis than the response ratio of nutrient acquisition. Harris et al. (1985) pointed out that C costs of N and P

Table 5
Response ratios of the rate of photosynthesis, leaf N and P mass fraction due to rhizobia symbioses.

Plant species	Rhizobia strain	Response ratio			Reference
		Photosynthesis	Leaf P	Leaf N	
Glycine max	*B. japonicum*	1.05 (0.95–1.16)	1.02 (0.95–1.10)	0.76 (0.73–0.79)	Brown and Bethlenfalvay (1987)
G. max					
6 weeks	*B. japonicum*	1.13 (0.85–1.48)	1.03 (0.78–1.35)	0.92 (0.70–1.22)	Harris et al. (1985)
9 weeks	*B. japonicum*	1.07 (0.81–1.42)	1.28 (0.97–1.69)	1.11 (0.84–1.46)	Harris et al. (1985)
Vicia faba					
Low N, 54 days	*R. leguminosarum* bv. *viciae*	1.85 (1.52–2.24)	1.13 (1.03–1.23)	1.47 (1.33–1.62)	Jia et al. (2004)
Low N, 63 days	*R. leguminosarum* bv. *viciae*	2.15 (1.54–3.00)	1.30 (1.18–1.43)	1.62 (1.49–1.75)	Jia et al. (2004)
High N, 54 days	*R. leguminosarum* bv. *viciae*	1.09 (0.93–1.29)	1.51 (0.77–2.97)	1.06 (1.01–1.10)	Jia et al. (2004)
High N, 63 days	*R. leguminosarum* bv. *viciae*	1.16 (1.05–1.28)	1.08 (0.99–1.18)	1.08 (1.03–1.14)	Jia et al. (2004)
Weighted average		1.28 (1.04–1.57)	1.13 (1.02–1.26)	1.11 (0.86–1.44)	

The response ratio was performed following the guidelines for a meta-analysis, considering the fixed model, as described by Gurevitch and Hedges (2001). The significance of the response ratios was calculated with the statistical software package MetaWin 2.0 (Sunderland, MA, USA, Sinauer Associates). The meta-analysis considered the use of mean, standard deviation and replicates of both control and treatment. The weighted average considers that larger studies are counted more heavily than smaller studies. The equation is: Response Ratio = (Value *i* in symbiotic plant/Value *i* in non-symbiotic plant), where *i* may be the photosynthetic rate (on leaf area basis), the leaf N or the leaf P mass fraction. The values in parenthesis represent the confidence interval at 95% probability.
Harris et al. (1985) did not report the variability of their observations and therefore, based on the fact that work of Jia et al. (2004), who reported a variability of 6%, we assumed that 10% would not underestimate the real variability. This approach has been used by other authors (Ostonen et al., 2007) to overcome lack of data.

Table 6
Response ratios in the rate of photosynthesis, leaf N and P mass fraction due to AMF symbioses.

Legume	AMF	Response ratio			References
		Photosynthesis	Leaf P	Leaf N	
Glycine max	*G. mosseae*	1.17 (1.06–1.30)	0.73 (0.67–0.79)	0.87 (0.83–0.92)	Brown and Bethlenfalvay (1987)
G. max					
6 weeks	*G. fasciculatum*	1.09 (0.83–1.44)	1.78 (1.35–2.35)	1.32 (0.92–1.61)	Harris et al. (1985)
9 weeks	*G. fasciculatum*	1.06 (0.80–1.40)	1.01 (0.77–1.34)	1.11 (0.93–1.62)	Harris et al. (1985)
Vicia faba					
Low N, 54 days	unknown	1.69 (1.34–2.14)	1.40 (1.29–1.53)	1.06 (0.96–1.17)	Jia et al. (2004)
Low N, 63 days	unknown	2.00 (1.43–2.80)	1.66 (1.52–1.80)	1.16 (1.08–1.24)	Jia et al. (2004)
High N, 54 days	unknown	1.06 (0.90–1.26)	1.75 (0.87–3.52)	1.03 (0.98–1.08)	Jia et al. (2004)
High N, 63 days	unknown	1.09 (0.91–1.31)	1.35 (1.22–1.50)	1.04 (0.99–1.10)	Jia et al. (2004)
Phaseolus vulgaris					
Low P, 17 days	*G. etunicatum*	1.17 (1.10, 1.25)	0.94 (0.72, 1.25)	1.02 (0.78, 1.35)	Mortimer et al. (2008)
Low P, 24 days	*G. etunicatum*	1.14 (1.01, 1.28)	0.93 (0.71, 1.23)	1.10 (0.84, 1.46)	Mortimer et al. (2008)
Low P, 31 days	*G. etunicatum*	1.22 (1.13, 1.31)	1.00 (0.76, 1.32)	1.05 (0.80, 1.39)	Mortimer et al. (2008)
High P, 17 days	*G. etunicatum*	1.07 (0.99, 1.17)	0.90 (0.68, 1.19)	0.97 (0.73, 1.28)	Mortimer et al. (2008)
High P, 24 days	*G. etunicatum*	0.94 (0.82, 1.09)	0.98 (0.74, 1.29)	0.94 (0.71, 1.24)	Mortimer et al. (2008)
High P, 31 days	*G. etunicatum*	0.97 (0.86, 1.09)	0.98 (0.74, 1.29)	1.04 (0.79, 1.38)	Mortimer et al. (2008)
Trifolium repens					
Irrigated with water, 35 days	unknown	1.10 (1.04–1.17)	0.75 (0.46–1.23)	1.00 (0.68–1.48)	Wright et al. (1998b)
Irrigated with water, 50 days	unknown	1.24 (1.00–1.53)	0.67 (0.48–0.92)	0.50 (0.37–0.67)	Wright et al. (1998b)
Nutrient solution, 35 days	unknown	1.21 (0.83–1.77)	0.71 (0.53–0.96)	1.00 (0.48–2.10)	Wright et al. (1998b)
Nutrient solution, 50 days	unknown	1.27 (0.95–1.69)	0.40 (0.24–0.67)	0.64 (0.30–1.38)	Wright et al. (1998b)
Weighted average		1.14 (1.08–1.21)	1.06 (0.90–1.25)	1.00 (0.92–1.08)	

For definition see Table 5.
Mortimer et al. (2008) did not report the variability of their observations in leaf N and P mass fraction and therefore, based on the fact that work of Jia et al. (2004) reported a variability of 6%, we assumed that 10% would not underestimate the variability. This approach has been used by other authors (Ostonen et al., 2007) to overcome lack of data. Time periods mentioned from the work of Wright et al. (1998b) are approximate.

acquisition were higher in soybeans associated with rhizobia than in soybeans fertilized with N, but the total biomass of symbiotic and non-symbiotic soybean was similar at the end of the study (9 weeks). Higher costs of nutrient acquisition would imply lower biomass if the rate of photosynthesis would not have been increased. Indeed, Harris et al. (1985) suggested that C sink strength of symbioses stimulated the rate of photosynthesis.

The study of Jia et al. (2004) allowed a comparison of the effects of rhizobial and AM colonization on faba beans under low and high nutrient conditions. The rate of photosynthesis increased considerably due to rhizobia or AM fungi under low nutrient conditions. Under low nutrient conditions, both rhizobia and AM fungi individually (Table 6) or combined (Table 7) resulted in higher response ratios of photosynthesis than response ratios of nutrient acquisition (significant in the case of rhizobial plants). The poor response of photosynthesis to the inoculation of rhizobia and AM fungi under high nutrient conditions can be explained by down-regulation of the symbioses (Schulze, 2004; Bittman et al., 2006).

Wright et al. (1998b) demonstrated a consistent increase in the rate of photosynthesis of mycorrhizal white clover (*Trifolium repens* L.) compared with non-mycorrhizal plants, in both nutrient-poor and nutrient-amended conditions, during at least 55 days. Two data points from Wright et al. (1998b), from a series of eight observations, allow direct comparison of the nutrient mass fraction in the leaves between the two main treatments. The response ratios of photosynthesis were significantly larger than the response ratios of nutrient mass fractions in the symbioses at the later stage of plant development (Table 6). The authors emphasized the effects of C

Table 7
Response ratios of photosynthesis rates, leaf N and P mass fraction due to combined rhizobia and AM fungi symbioses.

Legume	Rhizobia + AMF	Response ratio			Reference
		Photosynthesis	Leaf P	Leaf N	
Glycine max	*B. japonicum* + *G. mosseae*	1.22 (1.12–1.33)	0.68 (0.62–0.75)	0.72 (0.68–0.77)	Brown and Bethlenfalvay (1987)
G. max					
6 weeks	*B. japonicum* + *G. fasciculatum*	1.23 (0.93–1.62)	1.83 (1.39–2.41)	1.22 (0.92–1.61)	Harris et al. (1985)
9 weeks	*B. japonicum* + *G. fasciculatum*	1.14 (0.86–1.50)	1.30 (0.99–1.72)	1.23 (0.93–1.62)	Harris et al. (1985)
Vicia faba					
Low N, 54 days	*R. leguminosarum* bv. *viciae*	2.46 (1.94–3.12)	1.54 (1.36–1.75)	1.64 (1.50–1.79)	Jia et al. (2004)
Low N, 63 days	*R. leguminosarum* bv. *viciae*	3.08 (2.24–4.22)	1.79 (1.68–1.92)	1.77 (1.63–1.91)	Jia et al. (2004)
High N, 54 days	*R. leguminosarum* bv. *viciae*	1.25 (1.03–1.51)	1.95 (1.00–3.82)	1.14 (1.08–1.20)	Jia et al. (2004)
High N, 63 days	*R. leguminosarum* bv. *viciae*	1.28 (1.12–1.47)	1.46 (1.35–1.58)	1.19 (1.14–1.25)	Jia et al. (2004)
Weighted average		1.51 (1.15–1.98)	1.41 (0.95–2.09)	1.23 (0.92–1.65)	

For definition see Table 5.
Jia et al. (2004) worked with an unknown AMF.

sink strength by AM associations on the photosynthetic metabolism of the plants. At the 14th day of the experiment, the rates of photosynthesis were more than 3.2 times higher in the mycorrhizal plants than in the non-mycorrhizal plants (Wright et al., 1998b). Further investigation revealed that the increase in the rates of photosynthesis was correlated with an increased expression of the enzymes cell wall invertase (E.C. 3.2.1.26) and sucrose synthase (E.C. 2.4.1.13) in the roots of mycorrhizal white clover, which reflected increases in the C sink strength of the mycorrhizal symbiosis (Wright et al., 1998a).

Also Mortimer et al. (2008) demonstrated that the rate of photosynthesis of common beans (*Phaseolus vulgaris* L.) was increased due to increased C sink strength of the AM symbioses (higher below-ground respiration), while there was little evidence of changes in nutrient mass fraction in the leaves. Table 6 presents the response ratios of the rate of photosynthesis and the nutrient accumulation in the leaves of common beans from that experiment. Although the statistical significance was not strong, there was a higher increase in the rate of photosynthesis than in the leaf nutrients, especially at low P supply.

Considering all data in a meta-analysis, photosynthetic rates were significantly increased by 28 and 14% due to rhizobial and AM symbioses, respectively, and 51% due to dual symbiosis (Tables 5–7). The leaf P mass fraction was increased significantly by 13% due to rhizobial symbioses. Although the increases were not significant when the confidence intervals are considered, the leaf P mass fraction was increased 6% by AM symbioses and 41% due to dual symbioses. The leaf N mass fraction increased by 13% due to rhizobial symbioses and 23% by dual symbioses.

It is important to note that the studies analysed in this paper were not originally designed to test sink stimulation, but they provided evidence that sink stimulation occurred to compensate the C costs of the extra nutrient acquired through the symbioses. In the studies listed in Tables 5–7, photosynthesis rates were expressed on a leaf area basis whereas nutrients were expressed on a dry weight basis. One implication is that photosynthesis expressed on a dry weight basis would differ slightly from that expressed on a leaf area basis (e.g. Harris et al., 1985). For example, AM symbiosis increased photosynthesis by 9 and 6% on a leaf area basis (Table 5), whereas it increased by 23 and 6% on a dry weight basis (Table 1) in the sixth and ninth week, respectively. However, we believe that variation will exist in the magnitude of the photosynthetic responses depending on the unit of measurement considered, but that the trends are similar.

We calculated the additive effects by summing the responses of only nodulated or only AM plants as compared to non-inoculated plants, to obtain an estimation of dual symbioses on the rate of photosynthesis (Table 8). When comparing the calculated rates with the observed rates, we conclude that rate of photosynthesis is increased in an additive way when plants form combined symbioses. Should sink stimulation turn out to be less than additive, combined symbioses would compromise plant performance and productivity in order to maintain their own C supply, unless negative interactions between the symbionts would dominate during a phase when both are well-established.

2.5. Is the magnitude of sink stimulation by rhizobia and AM symbioses the same?

The magnitude of sink stimulation of photosynthesis will depend on the intrinsic regulation of the symbioses, according to plant nutritional demands and the developmental stage of the plant.

2.5.1. Ontogeny of C sink strength of rhizobial symbiosis

Sink stimulation of photosynthesis is likely to follow the increase in the C sink strength of symbioses. The C sink strength in rhizobial associations is determined to a large extent by the rate of N_2-fixation. The reason is that N_2-fixation requires a large amount of energy provided by intense oxidative phosphorylation by bacteroids (Minchin et al., 1981; Atkins, 1984; Minchin and Witty, 2005) whereas the costs for growth and maintenance of nodule biomass vary little throughout the plant cycle (Witty et al., 1983; Ryle et al., 1984; Voisin et al., 2003).

Nodule development and N_2-fixation are regulated throughout plant development, such that the highest rates of N_2-fixation in various crops take place in the period from flowering to early pod filling when N demand is greatest (Lawn and Brun, 1974; Bethlenfalvay and Phillips, 1977; Ryle et al., 1984; Warembourg and Fernandez, 1985; Hungria and Neves, 1986; Senaratne and Ratnasinghe, 1993). Zapata et al. (1987) suggested that plants may meet N demand from the soil or from N fertilizer in the beginning because the demand is low and N uptake is effective, but they have to rely on N_2-fixation after flowering. During these periods, efficient

Table 8
The additive effects of rhizobia and AM fungi on photosynthesis.

Plant species	Unity	Control (C)	AMF (M)	Rhizobia (R)	Rhizobia + AMF		Reference
					Observed	Estimated	
Glycine max	μmol CO_2 m^{-2} leaf $s^{-}c^{1}$	15.50	18.00	16.00	18.50	18.50	Brown and Bethlenfalvay (1987)
Vicia faba							
5 weeks	mg ^{14}C g^{-1} shoot C h^{-1}	7.02 c	7.60 b	7.92 b	8.23 a	8.50	Kucey and Paul (1982b)
6 weeks	mg ^{14}C g^{-1} shoot C h^{-1}	6.79 c	6.96 b	7.32 b	9.24 a	7.49	Kucey and Paul (1982b)
5 weeks	mg $^{14}CO_2$ g^{-1} shoot C h^{-1}	17.40	18.80	18.20	20.20	19.60	Paul and Kucey (1981)
Cajanus cajan							
25 days	^{14}C-activity (Bq mg^{-1})	1.40 a	1.95 bc	1.55 ab	2.00 c	2.10	Sivaprasad and Rai (1985)
35 days	^{14}C-activity (Bq mg^{-1})	2.80 a	4.60 c	4.05 b	5.40 d	5.85	Sivaprasad and Rai (1985)
60 days	^{14}C-activity (Bq mg^{-1})	0.65 a	1.00 bc	0.02 ab	1.10 c	0.37	Sivaprasad and Rai (1985)

The equation for additive effects is described as: Additive effects = (M) + (R) − (C), where (M), (R) and (C) are the rates of photosynthesis in mycorrhizal plants, rhizobial plants and control plants (without any symbiosis), respectively.
Statistics are presented as in each original study. Different letters in the same row are significantly different values at $P < 0.05$. In the data from Paul and Kucey (1981), the rates in AMF and Rhizobia + AMF are higher at $P < 0.05$ and Rhizobia are higher at $P < 0.10$ in relation to the control.
The data from Sivaprasad and Rai (1985) was converted with the equation Bq mg^{-1} = CPM/60.20, where CPM is the ^{14}C counting of atom decays per minute per sample, which was divided by 60 (seconds per minute) becomes Bq. Bq is divided by 20, because that is the weight of the sample in milligrams.
The correlation of observed and estimated rates with rhizobia + AMF is $R^2 = 0.995$ with $P < 0.01$ for the two tailed test, which indicates that the effects are additive and not synergistic.

nodulation is essential and sink stimulation of photosynthesis occurs. As rates of N_2-fixation are maintained at high energetic costs, C limitation would represent a threat for the success of rhizobial symbioses. High-yielding varieties associated with highly efficient N_2-fixing rhizobial strains have a relatively high activity of nodule phosphoenolpyruvate carboxylase (PEPC; E.C. 4.1.1.31) (Atkins, 1984), which might indicate a need for increasing the C uptake. While the presence of enzymes such as uptake hydrogenase (E.C. 1.12.7.2; Hungria et al., 1989) and PEPC (Atkins, 1984) recycles some of the energy in the nodule, C limitation is mainly resolved through an increased rate of photosynthesis.

High N availability in soil may lead to down-regulation of the nitrogenase activity and nodule viability (Schulze, 2004). The N-feedback mechanism presumes that N compounds in the phloem sap moving into nodules regulate the rate of N_2-fixation (Parsons et al., 1993). Hartwig (1998) reasoned that symbiotic N_2-fixation is regulated by the plant's N sink demand and suggested that nitrogenase activity is modulated by sensing the plant N:C ratio, possibly through phloem translocatable compounds. A reduced catabolism in the leaves also resulted in an increased concentration of ureides and amino acids, and therefore resulted in reduced nitrogenase activity and N_2-fixation (King and Purcell, 2005). A way of achieving such regulation may be related to a complex amino-acid cycling mechanism both determined by the plant and the rhizobia (Lodwig et al., 2003). Therefore, as sink stimulation is related to the process of N_2-fixation, down-regulation of N_2-fixation in the nodules would result in down-regulation of photosynthesis.

2.5.2. Ontogeny of C sink strength of AM symbiosis

The C sink strength of AM fungi is to a large extent determined by the growth and maintenance of both the intra- and extraradical mycelium (Johnson et al., 2002), particularly because mycelia accumulate a large amount of lipids, the synthesis of which is among the most energy-demanding of organic compounds (Table 3). The costs of P uptake through membranes are estimated to be similar in both hyphae and root tips, even though the P uptake system of AM fungi has a higher affinity than that of plants (Smith and Read, 2008). In annual crops, such as soybean and faba bean, the biomass of AM fungi follows a logistic growth curve, which increases up to plant flowering (Bethlenfalvay et al., 1982a, 1982b; Kucey and Paul, 1982a). After flowering, the fungi stop growing and require C only for maintenance (Kucey and Paul, 1982a), although they can continue to accumulate lipids (Bago et al., 2003). However, the relative costs of AM symbiosis are larger early in plant development when AM fungal colonization is indispensable for plants because the root system is still small and hyphae are more efficient in reaching P, which is poorly-mobile in soil (Grant et al., 2005; Bittman et al., 2006).

Root AM fungal colonization and photosynthate supply are correlated with the P concentration in the external growth medium (Peng et al., 1993; Olsson et al., 2002; Valentine and Kleinert, 2007), but there are no direct effects of the medium P concentration on the metabolism of extraradical hyphae (Olsson et al., 2002). In fact, photosynthate supply to AM mycelia is proportional to plant demand for P, with feed-backs in short-term alleviation of P stress (Valentine and Kleinert, 2007). Therefore, plants with a higher leaf P mass fraction down-regulate the carbohydrate supply to the AM mycelia (Menge et al., 1978), comparable with the effects of phloem N concentration on nodulation. Under severe P limitation in the soil, increasing P supply by fertilization may favour the AM fungal colonization until the deficiency is alleviated (Bolan et al., 1984) because severe P deficiency limits photosynthesis, but AM fungal colonization will be reduced if P supply is further increased and plant growth is no longer limited by P (Peng et al., 1993; Bittman et al., 2006).

2.6. Does sink stimulation of leaf photosynthesis by symbioses increase yield?

Sink stimulation of leaf photosynthesis could increase yield if increased photosynthesis is productively used. There is evidence that both rhizobia and AM lead to changes in dry matter partitioning that affect the harvest index, the ratio seed yield:total plant dry weight. If an inoculated plant produces more seeds than the non-inoculated counterpart, and the harvest index is higher as well, it follows that less leaf area was made available to produce grains while the C costs with symbioses were compensated by the photosynthesis. It is important to note that, although large amounts of C in rhizobial symbioses are transferred to the nodule, 21–52% of the C first allocated to the nodules may be returned via the xylem as incorporated organic N, ureides or amides (Minchin et al., 1981). The cycling of amides and ureides to form proteins and other plant compounds in nodulated plants may save a part of the newly assimilated C, which in turn supports the formation of extra plant biomass.

Conversely, AM fungi use the C allocated to build their own biomass, and consume more C in maintenance respiration than for nutrient uptake. In that case, sink stimulation by the carbon sink strength of AM symbioses may not result in higher plant biomass, because higher photosynthesis may be accompanied by increased root symbiont respiration (Paul and Kucey, 1981; Harris et al., 1985; Johnson et al., 2002; Valentine and Kleinert, 2007; Mortimer et al., 2008). Furthermore, the P taken up by hyphae, converted to polyphosphates, and transported in motile vacuoles until P can be transferred to the root vessels with the aid of transporter proteins (Smith and Read, 2008). That suggests that P transfer from fungal hyphae to the plant does not result in increased C availability for plant growth.

There is evidence that rhizobial symbiosis increases legume grain yields by increasing the harvest index. Kantar et al. (2003) noted that the largest values of harvest index were well correlated with greater numbers of nodules and Xavier and Germida (2002, 2003) demonstrated that larger harvest indices were correlated with increased total N content in the shoot, which means that plants performed better under symbiotic conditions.

Positive effects of an AM association on yield and harvest index seem to depend on the plant–AM fungal association. In experiments with soybean (Ross, 1971; Kuo and Huang, 1982), all AM fungal species stimulated an increase in yield and harvest index, but in experiments with lentil (*Lens culinaris* Medikus) and pea (Xavier and Germida, 2002, 2003) positive effects of AM colonization were not always evident. In fact, certain plant–AM fungal combinations are more successful than others in promoting plant growth (Smith and Read, 2008). Although several other factors are important for plant productivity (water, nutrients, soil physical properties, etc.), changes in the harvest index suggest that sink stimulation of photosynthesis by symbioses, in addition to the effects of improved plant nutrition, could have consequences for crop yields.

Furthermore, sink stimulation of photosynthesis could possibly lead to an increased period of leaf activity or delayed senescence (Paul and Peliny, 2003), which in turn could increase the potential period for plant growth and grain filling. Paul and Peliny (2003) stated that higher photosynthesis prior to the early phase of senescence may actually lead to a longer photosynthetically-active life of leaves. Indeed, Ono et al. (2001) showed that when the demand for carbohydrates is weak, leaves accumulate sugars and start to senesce. Conversely, low leaf sugar concentration leads to an increase in photosynthesis or to delayed leaf senescence. In soybeans, the synergistic effects of prolonged N acquisition and the stimulation of photosynthesis by the rhizobia symbioses postponed

the degradation of leaf protein and chlorophyll (Abu-Shakra et al., 1978), which could result in larger yields. The effects of AM symbiosis on leaf senescence await more detailed investigation.

2.7. Is sink stimulation by rhizobia and AM symbioses quantifiable, or does it remain a theoretical concept?

The patterns of the Tables 5–7 give reasonable evidence for a strong effect of rhizobia and mycorrhizal symbioses on the rates of photosynthesis, which goes beyond the influence of nutrient acquisition. However, given the low number of published studies, containing comparable measurements of photosynthesis and leaf nutrients, further experimental testing is clearly required. Sink stimulation has been identified as one possible explanation for the differences in responses between nutrient-fertilized plants and symbiotic plants by many authors (e.g. Pang and Paul, 1980; Harris et al., 1985; Wright et al., 1998a, 1998b; Mortimer et al., 2008), but the order of magnitude of this phenomenon has not been measured accurately. We realize that testing sink stimulation of photosynthesis by the carbon sink strength of symbioses raises several difficulties, particularly because it is difficult to determine the linear relationships between C costs and the sink stimulation of photosynthesis. In fact, to test sink stimulation, we should ensure symbiotic and fertilized plants with similar size at same developmental stage. Additionally, we should be aware of changes in the nutrient metabolism of symbiotic and fertilized plants and the root mass and activity. In the case of mycorrhizal symbioses, under stress conditions other than nutrient limitations (i.e. drought, heavy metals, etc.), fungi may also play a protective role in plant physiology, although their relative costs:benefits are difficult to measure (Fitter, 1991).

One way to test sink stimulation of photosynthesis is by measuring response curves of photosynthesis of nutrient-fertilized and symbiotic plants and identifying accurately the physiological mechanisms (von Caemmerer, 2000) that regulate the rates of photosynthesis under symbiotic conditions. Hopefully, those measurements will strengthen our understanding of how much sink stimulation is relevant throughout the plant cycle. If the C sink strength would be quantifiable, it should be expressed in terms of photosynthesis limitation due to triose-P export from the chloroplasts (e.g. Sharkey, 1985; Harley and Sharkey, 1991; Harley et al., 1992; von Caemmerer, 2000).

2.8. Towards better models of photosynthesis by including sink stimulation

If the phenomenon of sink stimulation is included in simulation models of photosynthesis, we could analyse to what extent photosynthesis can be increased to compensate for C costs of symbioses and we could assess possible changes in the harvest index of crops due to symbiotic associations. In natural ecosystems, sink stimulation of photosynthesis by rhizobial and AM symbioses would be observed in well-irradiated environments, such as savannas, grasslands or forests of pioneer plant species rather than in closed dense forests, because sink stimulation requires that photosynthesis is not primarily limited by light, water and CO_2. Cropping systems, specialized systems subjected to different management which may affect the symbioses (Hungria et al., 2005; Cardoso and Kuyper, 2006), may take advantage of the sink stimulation effect because it may improve yields in an efficient way. However, estimating the magnitude of sink stimulation under field conditions will remain a hard task. It is therefore necessary to improve models to assess the possibilities of manipulating sink stimulation of photosynthesis in favour of higher yields.

3. Conclusions

Whereas sink stimulation of photosynthesis as a function of strong sinks in the plants (i.e. fruits, storage organs and seeds) has been described previously (Herold, 1980; Paul and Foyer, 2001), in this paper we extended the concept to the C sink strength of root symbioses on the rates of photosynthesis. The C sink strength of rhizobial symbioses is mainly related to the respiration associated with rates of N_2-fixation, whereas the C sink strength of AM symbioses is mostly associated with the growth respiration of mycelium. The C sink strength of both symbioses is regulated according to the nutritional demand of the plant. We identified three potential manifestations of sink stimulation of photosynthesis by the C sink strength of rhizobia and AM symbioses: increased photosynthetic nutrient efficiency, increased harvest index and delayed leaf senescence. Increased nutrient acquisition through rhizobial and AM symbioses does not fully explain the increase in the rates of photosynthesis of symbiotic legumes. Increased photosynthetic nutrient efficiency and harvest index seem to be equally important in the two symbioses, whereas delayed leaf senescence has not been observed in AM symbiosis.

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