Chapter 12
Carbon Metabolism and Costs of Arbuscular Mycorrhizal Associations to Host Roots

Alex J. Valentine, Peter E. Mortimer, Aleysia Kleinert, Yun Kang, and Vagner A. Benedito

12.1 Introduction

The symbiotic relationship of plants with mycorrhizal fungi is cited as being a driving force behind the shift of plants to land, with the hyphal network enabling improved uptake by poorly developed roots in nutrient-poor conditions. This 470-million-year-old collaboration is a widespread phenomenon with 70–90 % of terrestrial plant species colonized by arbuscular mycorrhizal fungi. The evolutionary history of this relationship is supported by both fossil records and the fungal recognition mechanism that are conserved among plants. The premise of the symbiosis is the reliance of the fungi on photosynthetically derived carbohydrates supplied by the plant in exchange for improved nutrient (predominantly phosphate) and water uptake mediated by the extensive hyphal network. Recent studies have shown that the flow of carbon to the fungus can be downregulated under sufficient nutrient regimes and that the fungus is also able to control the transfer of nutrients to

A.J. Valentine (✉) • A. Kleinert
Botany and Zoology Department, Faculty of Science, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa
e-mail: alexvalentine@mac.com; kleinert@sun.ac.za

P.E. Mortimer
World Agroforestry Centre (ICRAF), China and East Asia Node, c/o: Kunming Institute of Botany, Kunming 650204, China
e-mail: peter@mail.kib.ac.cn

Y. Kang
Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA
e-mail: ykang@noble.org

V.A. Benedito
Genetics and Developmental Biology Program, Division of Plant and Soil Sciences, West Virginia University, 2090 Agricultural Science Building, Morgantown, WV 26506, USA
e-mail: vagner.benedito@mail.wvu.edu

R. Aroca (ed.), *Symbiotic Endophytes*, Soil Biology 37,
DOI 10.1007/978-3-642-39317-4_12,

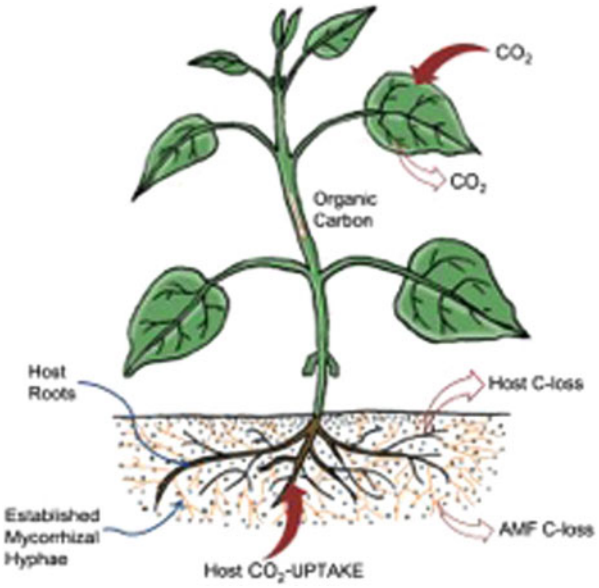

Fig. 12.1 The carbon costs of the arbuscular mycorrhizal root system, expressed as C inputs and losses above and below ground. Aboveground C inputs are via photosynthetic CO_2 assimilation and the losses are via respiratory CO_2 release. Belowground costs include CO_2 losses from host root and fungal symbiont respiration, due to tissue growth and nutrient acquisition. The refixation of CO_2 from the rhizosphere may replenish some of the respiratory CO_2 losses. Additionally, the increased translocation of organic C from the aboveground to belowground tissues may also incur a C cost (Figure provided by Dr. Yun Kang)

less than beneficial hosts (Selosse and Rousset 2011; Maillet et al. 2011; Kiers et al. 2011). This points to a biological "market" with reciprocal rewards to stabilize mycorrhizal symbiosis.

12.2 The C Cost of Arbuscular Mycorrhizae

AM fungi are dependent on the host plant as a C source and therefore act as a C sink. Once established, the fungal metabolic activities and growth can promote carbon sink stimulation of photosynthesis from the host plant (Fig. 12.1). It has been estimated that the fungus receives between 10 and 23 % of the plant's photosynthetically fixed carbon (Snellgrove et al. 1982; Kucey and Paul 1982; Koch and Johnson 1984; Harris et al. 1985; Jakobsen and Rosendahl 1990). Black et al. (2000) showed that mycorrhizal plants have a higher photosynthetic rate than non-mycorrhizal plants. This may be because of either an increased level of phosphate in the leaves due to the mycorrhizae (Azcon et al. 1992; Black et al. 2000) or because the AM fungus acts as a carbon sink (Snellgrove

et al. 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990). Both explanations have been found to be true but under different conditions and for different plants. Therefore it may result from a combination of both, depending on the growing conditions and the developmental stage of both the fungus and the plant.

In particular, the developmental stage of the symbiosis may warrant further exploration in order to gain a more accurate account of the C costs involved. Although the percentage AM colonization may be used as a means of estimating the potential mycorrhizal sink, there is conflicting evidence as to whether or not the percentage colonization of the root by AM fungi is related to the soluble carbohydrate content of the root. Pearson and Schweiger (1993) found that colonization was negatively correlated with the soluble carbohydrate content of the root, while Thompson et al. (1990) found a positive correlation. This may be because the conflicting experiments were carried out during different developmental stages of colonization. The three stages of colonization are the lag phase, the phase of rapid development, and the plateau phase (Smith and Read 1997). Pearson and Schweiger (1993) carried out their experimental work towards the end of the phase of rapid development, when the colonization period starts to decline and therefore is a subsequent decline in the demand for C by the fungus. Thompson et al. (1990) experimented during the end of the lag phase and the start of the phase of rapid development, when the demand for C is high. It appears that the process of colonization does depend on carbohydrates from the root during the initial phases and then reaches equilibrium as the process of colonization comes to an end and a stable symbiotic relationship develops. The carbon that is taken up by the fungus is incorporated into the growth and development of new fungal structures and spores. More than 90 % of the root can be colonized by an AM fungus (Motosugi et al. 2002) and can constitute up to 20 % of the root dry mass (Harris and Paul 1987).

Respiration of colonized roots was found to be between 6.6 and 16.5 % (depending on fungal species) higher than non-colonized roots in cucumber plants (Pearson and Jakobsen 1993). The increased respiration rate contributes to the sink effect of the fungus and indicates that colonized roots have a higher metabolic activity than non-colonized roots. This may in part be due to the losses incurred by the symbiont itself. There are three main ways that organic C is lost from the host via the fungus (Fig. 12.1): firstly via the loss of sloughed off fungal material, secondly through the release of fungal spores into the soil, or thirdly via the exudation of organic acids and phosphatase enzymes by the fungus. Fungal mycelia are constantly being replaced because of older material either breaking off as the root pushes through the soil or dying and being released into the soil. Bethlenfalvay et al. (1982a, b) found that as much as 88 % of the fungal biomass was external of the root for soybean; similarly Olsson and Johansen (2000) found that 70 % of the fungal biomass was external mycelium on cucumber roots. This will account for a large portion of C lost into the soil considering that at some stage the external hyphae will be released into the soil. The release of spores from the external mycelium accounts for a high percentage of lost organic C. In a study done by

Sieverding (1989), it was estimated that 919 kg ha^{-1} of plant C went into the production of spores, which are subsequently released into the soil by the fungus. Furlan and Fortin (1977) found spore production was influenced by the amount of C that is available to the fungus. The third means of organic C loss is through the exudation of organic acids and phosphatase enzymes by the fungal hyphae in order to aid in the uptake of nutrients such as phosphate. However, the main body of evidence supporting this has been found in ectomycorrhizae (Bolan et al. 1987). Although the release of organic acids is not thought to be the primary means of P uptake (Bolan 1991), it does constitute a loss of organic C derived from the host.

12.3 Arbuscular Mycorrhizal Efficiency and C-Flux Estimation

The transfer of fixed C from the host to the symbiont has a direct effect on the host plant and thus it is important to quantify this process. Koide and Elliott (1989) described this relationship mathematically using various models to describe both the gross benefit of the mycorrhizal colonization and the net benefit of colonization.

Gross benefit was defined as the difference between the quantity of gross C assimilation (mole C) in mycorrhizal and non-mycorrhizal plants over a given period of time (Koide and Elliott 1989):

$$\Delta A_{\mathrm{m}}^{\mathrm{g}} - \Delta A_{\mathrm{nm}}^{\mathrm{g}}$$

where $\Delta A_{\mathrm{m}}^{\mathrm{g}}$ and $\Delta A_{\mathrm{nm}}^{\mathrm{g}}$ are the gross C assimilation of the mycorrhizal and non-mycorrhizal plants during that time interval, respectively.

The net benefit of colonization for the same time period was described as the difference between mycorrhizal and non-mycorrhizal C accumulation (moles C) in the whole plant over the given time period (Koide and Elliott 1989):

$$\Delta C_{\mathrm{m}}^{\mathrm{w}} - \Delta C_{\mathrm{nm}}^{\mathrm{w}}$$

where $\Delta C_{\mathrm{m}}^{\mathrm{w}}$ and $\Delta C_{\mathrm{nm}}^{\mathrm{w}}$ represent the amount of C accumulated in the mycorrhizal and non-mycorrhizal plants over the given time period.

Koide and Elliott (1989) also described the efficiency of the relationship in terms of P acquisition, P utilization, and belowground *C* utilization. The efficiency of the P acquisition was defined as

$$\frac{\Delta P^{\mathrm{w}}}{\Delta C^{\mathrm{b}}}$$

where ΔP^{w} is the total P that has accumulated in the plant during the given time interval and ΔC^{b} is the total belowground C expenditure over the same time period

(Koide and Elliott 1989). This describes the efficiency of the relationship in terms of the amount of P taken up compared to the amount of C used for the uptake of P. C^{b} can be calculated as follows (Koide and Elliott 1989):

$$C^{b} = C^{r} + C^{o} + C^{n}$$

where C^{r} is the C that is allocated to the root tissue, C^{o} is the C lost via root belowground respiration, and C^{n} is the nonrespiratory, belowground C loss.

The efficiency of P utilization was defined by the following equation (Koide and Elliott 1989):

$$\frac{\Delta C^{w}}{\Delta P^{w}}$$

where ΔC^{w} is the total amount of C accumulated, in the whole plant, over the same period (Koide and Elliott 1989). This efficiency can be applied to any of the respective plant components. The final model proposed by Koide and Elliott (1989) was used to define the efficiency of belowground C utilization and was expressed as the ratio ΔC^{w}:ΔC^{b}. This ratio is the product of the previous two models:

$$\frac{\Delta C^{w}}{\Delta C^{b}} = \frac{\Delta C^{w}}{\Delta P^{w}} \times \frac{\Delta P^{w}}{\Delta C^{b}}$$

Koide and Elliott (1989) defined C^{b} (see above) as the total belowground C expenditure, which included all the C in the living tissue of the root system and the C lost from the root, via exudation, leaching, respiration, cell death, and direct transport to the fungus. Jones et al. (1991) went one step further and formulated two models that defined C^{b} in terms of the factors influencing the changes in C^{b}.

(a) The first model expressed C^{b} as a function of the C fixed via photosynthesis:

$$C_{b(Pn)} = \mathrm{Pn}\frac{\%C_{BG}}{100 - \%C_{SR}}t$$

where $C_{b(Pn)}$ is the amount of photosynthetically fixed C that is allocated below ground in a given period of time. Pn is the net photosynthetic rate as mmol C s^{-1} for the whole shoot system; $\%C_{BG}$ is the percentage of the total fixed C which is allocated below ground, over a given period of time; $\%C_{SR}$ is the percentage of the fixed C which was released via respiration in the shoot; and t is the length of the daily light period, measured in seconds. The term $100-\%C_{SR}$ represents the total amount of C left after respiration.

(b) Their second model expressed C^{b} as a function of the change in shoot mass, which will give an indication of C fluxes within the shoot:

$$\Delta C_{\mathrm{b(Wt)}} = \Delta W_{\mathrm{s}} \frac{\%C_{\mathrm{BG}}}{\%C_{\mathrm{ST}}}$$

where ΔW_{s} is the mean increase in shoot weight over a given time period and $\%C_{\mathrm{ST}}$ and $\%C_{\mathrm{BG}}$ are the mean percentages of the C fixed and allocated to the shoot tissue and to the belowground components, respectively.

The work of Koide and Elliott (1989) forms the backbone of mycorrhizal efficiency modeling, but they never tested their models experimentally. Therefore they have not defined the influencing factors that affected each of the parameters involved in the different models. The models proposed by Jones et al. (1991) elaborated on those of Koide and Elliott (1989) by defining C^{b} as a function of its influencing factors, not just its components.

However, the expression of C^{b} in terms of photosynthetically fixed C can be misleading. It assumes that photosynthetic C is the only source of C available to the plant. It does not include structural and nonstructural C that is already stored in the plant, which may be used and transported below ground or anywhere else in the plant for that matter. Similarly the expression of C^{b} in terms of the changes shoot mass assumes that the shoots are the only structures that will have an influence on belowground C, again ignoring other preexisting sources of C within the plant. This also neglects to take into account that AM and non-AM plants may allocate photosynthetic C in different proportions to different organs (Smith 1980; Koide 1985). Furthermore, these models do not include the stimulation of photosynthesis by mycorrhizal symbioses and, for this reason, may result in a misrepresented estimation of the carbon costs of mycorrhizal colonization.

12.4 Tissue Construction Cost and Belowground Respiration

Williams et al. (1987) proposed a model that can be used to determine the construction cost of various tissues within a plant. They defined construction cost as the amount of glucose required to provide C skeletons, reductant, and ATP for synthesizing the organic compounds in a tissue via standard biochemical pathways. They calculated tissue construction cost as

$$C_{\mathrm{w}} = \left\{ (0.06968 \times \Delta H_{\mathrm{c}} - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24} \right\} \frac{1}{E_{\mathrm{g}}}$$

where C_{w} is the construction cost of the tissue (g glucose g DW^{-1}) and ΔH_{c} is the ash-free heat of combustion of the sample (kJ g^{-1}). A is the ash content of the sample (g ash g DW^{-1}); k is the reduction state of the N substrate (NO_3 was used, therefore k is +5) and E_{g} is the deviation of growth efficiency from 100 %. E_{g} represents the fraction of the construction cost that provides reductant that is not

incorporated into biomass. Williams et al. (1987) determined the value of E_g to be 0.89.

Peng et al. (1993) slightly modified this equation and converted the g glucose into mmol C:

$$C_w = \left\{(0.06968 \times \Delta H_c - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24}\right\} \frac{1}{0.89} \times \frac{6,000}{180}$$

The units of construction cost are now mmol C g DW^{-1}. However, the tissue construction cost equation was further modified by Mortimer et al. (2005):

$$C_w = [C + kN/14 \times 180/24]\ (1/0.89)(6,000/180)$$

where C_w is the construction cost of the tissue (mmol C g DW^{-1}), C is the carbon concentration (mmol C g^{-1}), k is the reduction state of the N substrate, and N is the organic nitrogen content of the tissue (g/g DW) (Williams et al. 1987). The constant (1/0.89) represents the fraction of the construction cost which provides reductant that is not incorporated into biomass (Williams et al. 1987; Peng et al. 1993) and (6,000/180) converts units of g glucose/g DW to mmol C g DW^{-1}.

Peng et al. (1993) use the construction cost to determine the growth respiration, which was defined as the respired C associated with the biosynthesis of new tissue:

$$R_{G(t)} = C_t - \Delta W_c$$

where $R_{G(t)}$ is the growth respiration (ìmol CO_2 d^{-1}); C_t (ìmol CO_2 d^{-1}) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (ΔW_w, mg DW d^{-1}) by tissue construction cost (C_w). ΔW_c (ìmol d^{-1}) is the change in root C content and was calculated by multiplying the root C content and the root growth rate (ΔW_w, mg DW d^{-1}).

12.5 Arbuscular Mycorrhizal Carbon Costs in Dual-Symbiotic Roots

Arbuscular mycorrhizal (AM) hosts plants are also able to form dual root symbioses with AM and nitrogen-fixing symbionts. In particular, the ability of legumes to form tripartite associations with AM fungi and rhizobia gives them access to sources of P and N that would normally not be available to the plant. Rhizobia are able to fix atmospheric N and convert it into an organic form which is subsequently made available to the host (Lodwig and Poole 2003). The AM are not only more efficient in the uptake of P from the soil but can also access pockets of soil P that would ordinarily not be available to the host. This allows for improved plant growth, especially in nutrient-poor soils. The ability to form these

associations makes legumes valuable as a crop plant as it provides both the legumes and the subsequent crops with a renewable source of N and the ability to grow in low P soils (Frey and Schuepp 1992; Udvardi et al. 2005).

However, these nutrients provided to the host plant come at a cost; in exchange the host plant supplies the two symbionts with photosynthetically derived sugars (Vessey and Layzell 1987; Smith and Read 1997; Vance 2002). In spite of the C costs of maintaining a dual symbiosis, the cumulative benefits of dual inoculation are greater than those of singular inoculation to both the host plant and to the respective symbionts (Daft and El-Giahmi 1974; Cluett and Boucher 1983; Kawai and Yamamoto 1986; Pacovsky et al. 1986; Chaturvedi and Singh 1989). However the legume is able to balance these costs by increasing its photosynthetic rate, thus producing more sugars for the growth and maintenance of both itself and the two symbionts.

12.5.1 Symbiotic P and N Nutrition

One of the main functions of AM is the provision of soil P to the host plant; therefore, under low soil P conditions, the dependency of the host on the AM fungi increases (Smith and Read 1997). Fredeen and Terry (1988) found that AM-colonized legumes growing under low soil P had higher shoot P as well as greater shoot and nodule dry weights. This indicates the role that AM play in both the P nutrition and the growth of the host as well as the nodules. The enhancement of host growth is attributed to an increase in the production of photosynthate by the host, resulting from of the improved nutrition, obtained from the root symbioses (Fredeen and Terry 1988; Jia et al. 2004).

Rhizobia, found in the root nodules, play a crucial role in the N nutrition of their legume hosts. The bacteria are able to provide the host with reduced N, which is derived from atmospheric N_2. Atmospheric N is fixed into ammonia, with the aid of the enzyme nitrogenase, which is consequently exchanged with the host for C (Thorneley 1992; Lodwig and Poole 2003). Nodules are strong P sinks and the process of N fixation is energy intensive, resulting in the nodules requiring more energy and P than the host roots (Sa and Israel 1991; Al-Niemi et al. 1998; Almeida et al. 2000). Vadez et al. (1997) reported that nodules had a threefold greater concentration of P than other plant tissues, which gives an indication of the nodular sink strength for P. It has also been reported that a deficiency of P can lead to a reduction in both nodulation and symbiotic N fixation (Othman et al. 1991; Drevon and Hartwig 1997). Alternatively, P availability has been found to increase the ratio of nodule to total plant mass; nodule mass appears to be more influenced by the availability of P than nodule number (Othman et al. 1991; Almeida et al. 2000). This is confirmed by the work of Olivera et al. (2004), who reported that an increase in the P supplied to host plants led to a fourfold increase in nodule mass. This dependency on P by the nodule bacteria will also create a strengthened dependency

on AM fungi by the host in order to supply the high amounts of P required by the rhizobia.

Although legumes rely on the N contribution of the rhizobia for growth and development, the plant can access other sources of N. Numerous studies have shown that AM play an important role, both directly and indirectly, in the uptake of N and the subsequent supply of N to the host plant (Marschner and Dell 1994; Constable et al. 2001; Toussaint et al. 2004; Govindarajulu et al. 2005; Mortimer et al. 2009). This indirect effect of AM on host N nutrition is apparent in legumes. Mycorrhizal legumes have been reported to have a greater number of nodules, increased nodular weight, and improved N fixation rates, thereby enhancing the N nutrition of the host (Carling et al. 1978; Kawai and Yamamoto 1986; Luis and Lim 1988, Vesjsadova et al. 1993; Goss and de Varennes 2002; Mortimer et al. 2008). Another indirect means of AM influencing the N nutrition of host legumes can be seen in the study carried out by Mortimer et al. (2012); this study found that AM can counter the asparagine-induced inhibition of biologic nitrogen fixation (BNF; under N nutrition), thus allowing for the continued function nodules when exposed to an external source of N.

12.5.2 C Costs of the Dual Symbiosis

Root symbionts require C from the host plant in exchange for the nutrients provided, thus acting as C sinks. Therefore, by default they both compete for the same source of photosynthate from the host plant (Harris et al. 1985, Mortimer et al. 2008). This double sink created by the root symbionts can impose a considerable drain on host C resources, altering rates of photosynthesis and host growth. Numerous studies have revealed that the AM fungus receives between 10 and 23 % of host photosynthate (Snellgrove et al. 1982; Koch and Johnson 1984; Kucey and Paul 1982; Jakobsen and Rosendahl 1990) and the nodules between 6 and 30 % (Kucey and Paul 1981, 1982; Harris et al. 1985; Provorov and Tikhonovich 2003). Nodule number as well as BNF is influenced by the availability of photosynthate; therefore, any factors which influence the rates of photosynthesis will in turn influence the BNF process (Bethlenfalvay and Phillips 1977; Murphy 1986; Atkins et al. 1989; Sussanna and Hartwig 1996; Schortemeyer et al. 1999). As has been shown in the studies of Fredeen and Terry (1988) and Peng et al. (1993), when soil P and N are not limiting to plant development, a growth depression in the host plant and a decrease in nodular dry weight can occur. This is due to the C being used for symbiont growth in place of host growth (Fredeen and Terry 1988; Peng et al. 1993).

Despite this C drain imposed by the symbionts, both the rhizobia and the AM fungi generally result in the improved growth of the host plant; however, these growth benefits are most noticeable under low nutrient conditions, when the host plant is most reliant on the respective root symbionts. Numerous studies have highlighted the fact that the dual inoculation of the host plant by AM fungi and

nodule bacteria results in enhanced plant growth and symbiont development to a greater extent than singular inoculation (Daft and El-Giahmi 1974; Cluett and Boucher 1983; Kawai and Yamamoto 1986; Pacovsky et al. 1986; Chaturvedi and Singh 1989; Mortimer et al. 2009, 2012). Examples of this synergistic benefit resulting from dual inoculation are seen in the work of Nwoko and Sanginga (1999), who reported a 57 % increase in the percentage AM colonization of host plants, once the host was inoculated with *Bradyrhizobium*; similarly, the dual inoculation led to an increase in nodular weight. Furthermore, studies by Toro et al. (1998) and Jia et al. (2004) have shown that host plants had greater N fixation rates and that dual inoculation led to increased rates of photosynthesis and improved plant productivity.

As a means of compensating for the C being used by the two symbionts, the host plants increase its photosynthetic productivity, which is achieved in a number of different ways. Generally an increase in leaf P is attributed to the presence of AM; this can be used to fuel an increase the rate of photosynthesis; additionally, in a more indirect manner, host plants can increase the specific leaf area and rate of leaf expansion, further boosting photosynthetic productivity (Harris et al. 1985; Fredeen and Terry 1988; Jia et al. 2004). Harris et al. (1985) found 47 % increase in CO_2 fixation of nodulated soybeans and attributed this to the higher leaf P, improved mobilization of starch, and an increase in the specific leaf area of the soybeans. In the same way, Jia et al. (2004) have shown that plants with rhizobia and AM fungi have higher photosynthetic rates per unit leaf area. An alternative mechanism was provided by Fredeen and Terry (1988) who found that host plants had greater photosynthate production due to an increase in the rate of leaf surface expansion and not as a result of an increase in the rate of photosynthesis or photosynthesis on a leaf area basis. From the above studies, it is clear that host plants are able to compensate for the increased demand for photosynthate, resulting from the respective root symbionts, by a number of different mechanisms. This sink stimulation may be dependent upon the developmental stage of the root symbionts and their contributions to nutrient-based growth or uptake efficiencies.

12.5.3 Calculations for Dual Root Symbioses

Carbon and nutrient cost calculations for dual root symbioses in legumes may be calculated as follows:

(a) Specific P absorption rate (SPAR) (mg P g^{-1} root dw d^{-1}) or SNAR in the case of N, is the calculation of the net P absorption rate per unit root dw (Nielson et al. 2001):

$$\mathrm{SPAR} = (M_2 - M_1)/(t_2 - t_1) \times (\log_e R_2 - \log_e R_1)/(R_2 - R_1)$$

where M is the P content per plant and R is the root system (to include nodules, if nodulated) dry weight.

(b) Specific P utilization rate (SPUR) (g dw $mg^{-1}P\ d^{-1}$) or SNUR in the case of N is a measure of the dw gained for the P taken up by the plant (Nielson et al. 2001):

$$\text{SPUR} = (W_2 - W_1)/(t_2 - t_1) \times (\log_e M_2 - \log_e M_1)/(M_2 - M_1)$$

where M is the P content of the plant and W is the plant dry weight. The specific nitrogen utilization rate (SNUR) can be adapted from these equations to include N instead of P, as shown in Mortimer et al. (2008).

The benefits of using SPUR and SPAR for C cost analysis were evident in the comparison of nutritional benefits in terms of uptake and growth efficiency of legume hosts with dual symbiotic roots (Mortimer et al. 2008). In their study on the legume *Phaseolus vulgaris*, Mortimer et al. (2008) found that in the dual symbiosis, the AM colonization proceeded more rapidly than the nodulation when the plants were inoculated at the same time. The AM was the primary belowground sink of host C resources in the dual symbiosis with root nodules, which resulted in the delayed onset of nodular growth. This allowed for AM establishment and the subsequent enhancement of P nutrition, which benefited the later nodular and host development. AM colonization peaked at 17 days after inoculation; the decline in percentage colonization following this stage (24 days) coincided with the increase in nodule mass. It was argued that the increase in nodule development was delayed due to host C being used to support the high levels of AM colonization (thus allowing for increased P acquisition), confirmed by the higher construction costs and growth respiration of these plants. Once AM colonization reached the plateau phase, more C was available for nodule growth (Harris et al. 1985). Coupled with this argument is that nodules require relatively high amounts of P for normal growth and maintenance (Sa and Israel 1991; Al-Niemi et al. 1998; Almeida et al. 2000); thus under low P conditions, the host and nodules would rely on AM-derived P during the AM plateau phase. Therefore, initial AM development up to 17 days took preference over the formation of nodules, so that the plateau phase of AM was reached and the subsequent benefits of P nutrition accrued. This was also noticed in previous studies, where P nutrition was enhanced once the plateau phase of AM development was reached (Mortimer et al. 2005). This AM-induced response was primarily due to the improved P nutrition of AM roots, evidenced by the greater specific P absorption (SPAR) and utilization (SPUR) rates compared to non-mycorrhizal plants. These effects of P on host growth and nutrition are confirmed by previous studies showing improved P nutrition and growth of AM plants (Sanders and Tinker 1971; Smith 1982; Bolan 1991; Orcutt and Nilsen 2000). Confirmation for the preference of AM development over nodular growth (0–17 days) is apparent in the difference between colonization levels at low and high P and the fungal effect on the dual symbiotic belowground sink strength. This sink effect is evidenced by the higher

photosynthetic rates and root oxygen consumption rates of the double symbiotic roots at low P than at high P, for the period of 0–17 days. This is consistent with the work of Valentine and Kleinert (2007) who found that mycorrhizal plants had increased respiration resulting from the higher levels of root colonization associated with low P conditions.

Mortimer et al. (2008) also found that there were synergistic effects on nutritional physiology, owing to the dual symbiotic associations. These were most pronounced under low P conditions due to the key role of AM in the tripartite symbiosis. These synergistic effects on nodule development resulted in greater BNF by the nodules, providing the host with more N than their non-AM counterparts. This is consistent with previous studies showing enhanced levels of colonization and nodulation resulting from the synergistic effect of dual inoculation, as well as greater nodular dry weights (Daft and El-Giahmi 1974; Cluett and Boucher 1983; Kawai and Yamamoto 1986; Pacovsky et al. 1986; Chaturvedi and Singh 1989; Nwoko and Sanginga 1999). In addition, Catford et al. (2003) found that the tripartite symbiotic partners may autoregulate the development of the various symbionts. The nutritional benefits of the double symbiosis that resulted in greater host growth under low P are in agreement with the work of Gavito et al. (2000). Gavito et al. (2000) proposed that the improved nutrition of the mycorrhizal legumes led to enhanced N fixation, thereby resulting in the dual symbiotic plants having greater growth.

This cumulative sink effect imposes a considerable drain on host C reserves, as evidenced by the increased oxygen consumption and growth respiration of the dual symbiotic plants in study by Mortimer et al. (2008). The increased demand for host C by the two symbionts resulted in the host plants having higher photosynthetic rates, which concurs with the findings of Jia et al. (2004) for *Vicia faba*. Although Jia et al. (2004) attributed the higher photosynthetic rates to improved N and P nutrition, no evidence was presented for respiratory costs driving belowground sink stimulation of photosynthesis. In this regard, previous studies have found that colonization of host roots by AM led to increased levels of belowground respiration (Peng et al. 1993; Valentine and Kleinert 2007). Therefore, the addition of a further symbiont, such as nodule-producing N_2-fixing bacteria, should lead to an even greater respiratory demand on the host. From the current study, additional evidence for the greater C consumption by the dual symbiosis is the higher construction costs of these plants. This means that the hosts require more C for every gram of tissue produced, thus further increasing the photosynthetic and respiratory C costs. This is consistent with the work done by Peng et al. (1993) who found that mycorrhizal plants had higher construction costs than their non-mycorrhizal counterparts. These findings indicate a more prominent role for the AM in this tripartite symbiosis, because of its role in supplying P more effectively to both host and nodules, as well as contributing to the N economy of the host plant. Physiological and biochemical methods often involve large experimental procedures to determine the carbon costs of the root symbioses. The advent of quantitative tools of gene expression analysis may offer an efficient method to simplify these calculations.

12.6 Perspectives of Molecular Approach to Carbon Costs

The value of a molecular approach to complement the physiological costs assessment is evident in the study by Kiers et al. (2011), where the C allocation of gene expression in an AM symbiotic assemblage was traced as ^{13}C allocation in RNA. Not only is it important to determine the C costs of total gene expression but also to use transcriptional analyses to assess potential C fluxes during the symbiosis.

As described in the previous sections, the impact of nutritional root symbioses on the host's physiology is quite dramatic, not only in the root system but also as consequences to the functioning of aboveground organs. Higher respiratory demand of roots and organic C export imposes greater photosynthetic requirements from the shoots, while increased P (and N) availability to the shoots can lead to greater growth rates. The impact of this positive developmental-nutritional feedback between the participating symbionts goes beyond direct sink of primary C and N pathways in the shoot, such as photosynthesis, TCA cycle, N assimilation, and metabolite transport systems. Notwithstanding, the vast majority of the studies on gene expression of root symbioses concentrate on root tissues.

Using transcriptional analysis to quantify a specific cellular phenomenon is often complex because transcription is only one of many levels of gene expression regulation, including posttranscriptional and posttranslational levels. In metabolic pathways, many enzymes are commonly present in excess, so that variation in gene expression has little impact in the pathway flow, and key enzymes are often regulated by multiple mechanisms (e.g., mRNA degradation by small RNA targeting, translational arrest, protein phosphorylation, feedback allosteric regulation) to guarantee a precise metabolic regulation. Ideal genetic markers representing the carbon costs of the symbiosis from the plant host to the mycorrhizal fungus should present a specific and proportional regulation pattern of gene transcription. This may reflect the level of root infection and metabolic flow of organic C export. Furthermore, there is clearly a requirement for model experiments that correlate plant gene expression and metabolic carbon flow to the mycorrhizal symbiont. In this regard, a quantitative real-time (qPCR) approach comparing symbiotic systems with nonsymbiotic systems in both nutritionally sufficient and limiting conditions could be used in parallel with metabolic analyses (e.g., labeled carbon flow, adenylate energy charge status). This would immensely facilitate the assessment of plant energy costs in terms of reduced C flow to the mycorrhizal fungi. In addition, this would accrue further value if the genes could be assessed in aerial, photosynthetic organs of the plant.

A recent study on the impact of mycorrhization on gene expression in rice leaves revealed that this symbiosis increases pathogen resistance in the shoot by systemically inducing expression of defense-response genes, including diverse transcription factors (notably, AP2/EREBP and bHLH family members), calcium signaling and kinases (MPK6, CBP), and hormone-related genes, such as the jasmonic acid (JA) biosynthesis (Campos-Soriano et al. 2012). As demonstrated in tomato, JA is indeed an important plant hormone for mycorrhization and particularly in relation

to carbon partitioning. Prosystemin, a JA precursor, was shown to act as a positive effector of endomycorrhizal colonization in studies using genotypes defective or overexpressing prosystemin (Tejeda-Sartorius et al. 2008). Additionally, other hormones have been shown to be important for mycorrhizal association, such as strigolactone (Yoneyama et al. 2008), abscisic acid (Garrido et al. 2010; Rodriguez et al. 2010), and ethylene (Zsögön et al. 2008). It will be interesting to assess the impact of mycorrhizal symbiosis in the gene expression of shoots of other species and correlate the level of infection with transcriptional variations, in order to better characterize canonical gene expression patterns that are evolutionarily conserved in the plant kingdom.

Good genetic markers of mycorrhization have been described for pre-colonization (expansin EXBL1, specific WRKY transcription factors) and late stages of colonization (DXS-2, chitinase, glutathione S-transferase, β-1,3-glucanase, and an additional WRKY transcription factor) in several plant species (Dermatsev et al. 2010; Gallou et al. 2011). Additionally, the expression of the mycorrhiza-specific phosphate transporter PT4 (Harrison et al. 2002), the H^+-ATPase MTHA1 (Krajinski et al. 2002), and the mycorrhizal high-affinity monosaccharide transporter MST2 (Helber et al. 2011) can be used in quantitative experiments to assess relative mycorrhizal colonization in the root system.

Although the plant model *Arabidopsis thaliana* does not form mycorrhizal associations, the cross talk between the C and N pathways is mostly conserved among plant species, and the results found in *Arabidopsis* might serve to understand the physiological relations of C and N metabolism. A recent study identified two E3 ubiquitin ligases (ATL31 and ATL6) and two transcription factors (MYB51 and WRKY33) involved in both pathogen defense response and C/N metabolism (Maekawa et al. 2012). If future studies in other plants revealed that functional homologs of such genes have the same expression pattern, these genes might become suitable markers of mycorrhizal symbiosis and its impact on C/N physiology.

A comprehensive transcriptional analysis through microarray comparing gene expression profile between mycorrhizal roots and nonsymbiotic roots was performed in the model legume *Medicago truncatula*. Further confirmation by laser microdissection of cortical cells containing arbuscules revealed novel genes expressed specifically in infected cells (Gomez et al. 2009), including several membrane transporters (Benedito et al. 2010). Further studies confirmed and expanded this analysis, demonstrating specific plant and fungal genes during cellular reprogramming of the symbiosis in cortical root cells, including a potential Cu transporter, a H^+-dependent oligopeptide transporter, HAP5 and MYB transcription factors, a protease inhibitor, and a gene of unknown function in infected root cells, while the fungus showed expression changes in several genes, including upregulation of two 18S rRNA genes and downregulation of a RHO-protein GDI gene (Gaude et al. 2012). Importantly, Gomez et al. (2009) found genes specifically related to energy (ADP-ribosylation factor, ARF) and nitrogen metabolism (glutamine synthetase, arginase, ornithine aminotransferase) to be specifically expressed in colonized mycorrhizal root cortical cells, not to mention an ammonium

transporter (most likely involved in uptake of ammonium made available by the fungi) and, not surprisingly, several genes involved in lipid metabolism.

The arbuscule formation does indeed require a great deal of lipid synthesis by the plant cells to enable an enormous expansion of the plasma membrane to establish the symbiosome membrane that interfaces the arbuscule that allows chemical exchanges between symbionts. Trépanier et al. (2005) found that palmitic acid synthesis in the symbiotic fungus depends directly on sugar supply from the plant host and can synthesize 16-C fatty acids exclusively in intraradical hyphae. The host also needs a massive lipid biosynthesis, especially in the intraradically colonized cells, and this requirement should be counted as C cost of the symbiosis to the host.

CO_2-enriched atmosphere was shown to increase ectomycorrhizal association in roots of Pinus (Parrent and Vilgalys 2009). The authors used symbiotic plant genes to assess C and N flow (monosaccharide transporter and ammonium transporter, respectively) as well as the expression of fungal 18S rRNA to determine the level of association. Another approach to be considered in understanding carbon costs of mycorrhizal associations is genetics by the using of mutants. For example, three mutants defective in accumulation of starch in root cells of the model legume *Lotus japonicus* were used to assess the effect of root starch accumulation on mycorrhization (Gutjahr et al. 2011). Results pointed out that catabolism of root starch is an alternate route to feeding the symbiotic fungi; however, the authors challenge the hypothesis that plants feed the fungi only during the day, when photosynthetically active, but instead that root starch is used to feed the fungi with hexose at night. This is in agreement with the fact that an α-amylase gene is upregulated in mycorrhizal roots of *Medicago truncatula* (Gomez et al. 2009). It will be interesting to C costs to determine whether or not starch catabolism-related gene expression is regulated in a circadian rhythm pattern in mycorrhizal roots.

Another example of use of genetic tools to characterize carbon metabolism in the mycorrhizal symbiosis was the demonstration of the role of a sucrose synthase gene (MtSucS1) for arbuscule maturation and maintenance in *Medicago truncatula* (Baier et al. 2010). Antisense lines for this gene showed drastic developmental impairment as well as compromised mycorrhization, pointing out the specific role of sucrose synthase activity for this symbiosis.

Additionally, use of CO_2-enriched environments to compare plants under symbiotic and nonsymbiotic conditions using both metabolic and genetic approaches might lead to a more comprehensive understanding of how mycorrhizal fungi utilize plant's resources to grow. The use of mathematical modeling may be a useful resource to integrate genetic and physiological data in biotrophic mutualistic symbiosis.

12.7 Conclusions

The C costs of arbuscular mycorrhizal roots can be assessed using various scales of investigation, ranging from whole-plant physiological to biochemical to molecular biological. Moreover, it may be prudent to integrate these scales of investigation, in order to increase the depth of our understanding of the C costs of the arbuscular mycorrhizal symbiosis. Functional bookkeeping of C costs at various scales is obviously highly complex, and although we might develop approaches to calculate the net costs of this association, one can only appreciate the 400 million years that this symbiosis has been evolving on Earth for the benefit of both plant and fungus.

References

Almeida JPF, Hartwig UA, Frehner M, Nosberger J, Luscher A (2000) Evidence that P deficiency induces N feedback regulation of symbiotic N2 fixation in white clover (*Trifolium repens* L.). J Exp Bot 51:1289–1297

Al-Niemi TS, Kahn ML, McDermott TR (1998) Phosphorus uptake by bean nodules. Plant Soil 198:71–78

Atkins CA, Pate JS, Sanford PJ, Dakora FD, Matthews I (1989) Nitrogen nutrition of nodules in relation to "N-hunger" in cowpea (*Vigna unguiculata* L. Walp). Plant Physiol 90:1644–1649

Azcon R, Gomez M, Tobar R (1992) Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and phosphorous-fertilized plants of *Lactuca sativa* L. New Phytol 121:227–234

Baier MC, Keck M, Gödde V, Niehaus K, Küster H, Hohnjec N (2010) Knockdown of the symbiotic sucrose synthase MtSucS1 affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*. Plant Physiol 152:1000–1014

Benedito VA, Li H, Dai X, Wandrey M, He J, Kaundal R, Torres-Jerez I, Gomez SK, Harrison MJ, Tang Y, Zhao PX, Udvardi MK (2010) Genomic inventory and transcriptional analysis of *Medicago truncatula* transporters. Plant Physiol 152:1716–1730

Bethlenfalvay GJ, Phillips DA (1977) Effect of light intensity on efficiency of carbon dioxide and nitrogen reduction in *Pisum sativum* L. Plant Physiol 60:868–871

Bethlenfalvay GJ, Brown MS, Pacovsky RS (1982a) Relationships between host and endophyte development in mycorrhizal soybeans. New Phytol 90:537–543

Bethlenfalvay GJ, Pacovsky RS, Brown MS, Fuller G (1982b) Mycotrophic growth and mutualistic development of host plant and fungal endophyte in an endomycorrhizal symbiosis. Plant Soil 68:43–54

Black KG, Mitchell DT, Osborne BA (2000) Effect of mycorrhizal-enhanced leaf phosphate status on carbon partitioning, translocation and photosynthesis in cucumber. Plant Cell Environ 23:797–809

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants. Plant Soil 134:189–207

Bolan NS, Robson AD, Barrow NJ (1987) Effect of vesicular arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant Soil 99:401–410

Campos-Soriano L, García-Martínez J, Segundo BS (2012) The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. Mol Plant Pathol 13:579–592

Carling DE, Reihle WG, Brown MF, Johnston DR (1978) Effects of vesicular arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. Phytopathology 68:1590–1596

Catford JG, Staehelin C, Lerat S, Piché Y, Vierheilig H (2003) Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. J Exp Bot 54:1481–1487

Chaturvedi C, Singh R (1989) Response of chickpea (*Cicer arietinum* L.) to inoculation with *Rhizobium* and VA mycorrhiza. Proc Natl Acad Sci Ind B59:443–446

Cluett HC, Boucher DH (1983) Indirect mutualism in the legume- *Rhizobium* mycorrhizal fungus interaction. Oecologia 59:405–408

Constable JVH, Bassirirad H, Lussenhop J, Ayalsew Z (2001) Influence of elevated CO2 and mycorrhizae on nitrogen acquisition: contrasting responses in *Pinus taeda* and *Liquidambar styraciflua*. Tree Physiol 21:83–91

Daft MJ, El-Giahmi AA (1974) Effect of endogone mycorrhiza on plant growth. VII. Influence of infection on the growth and nodulation in French bean (*Phaseolus vulgaris*). New Phytol 73:1139–1147

Dermatsev V, Weingarten-Baror C, Resnick N, Gadkar V, Wininger S, Kolotilin I, Mayzlish-Gati E, Zilberstein A, Koltai H, Kapulnik Y (2010) Microarray analysis and functional tests suggest the involvement of expansins in the early stages of symbiosis of the arbuscular mycorrhizal fungus *Glomus intraradices* on tomato (*Solanum lycopersicum*). Mol Plant Pathol 11:121–135

Drevon JJ, Hartwig UA (1997) Phosphorus deficiency increases the argon- induced decline of nodule nitrogenase activity in soybean and alfalfa. Planta 201:463–469

Fredeen AL, Terry N (1988) Influence of vesicular-arbuscular mycorrhizal infection and soil phosphorous level on growth and carbon metabolism of soybean. Can J Bot 66:2311–2316

Frey B, Schuepp H (1992) Transfer of symbiotically fixed nitrogen from Berseem (*Trifolium alexandrinum* L.) to maize via vesicular-arbuscular mycorrhizal hyphae. New Phytol 122:447–454

Furlan V, Fortin JA (1977) Effects of light intensity on the formation of vesicular arbuscular endomycorrhizas on *Allium cepa* by *Gigaspora calospora*. New Phytol 79:335–340

Gallou A, Declerck S, Cranenbrouck S (2011) Transcriptional regulation of defence genes and involvement of the WRKY transcription factor in arbuscular mycorrhizal potato root colonization. Funct Integr Genomics 12:183–198

Garrido JM, Morcillo RJ, Rodríguez JA, Bote JA (2010) Variations in the mycorrhization characteristics in roots of wild-type and ABA-deficient tomato are accompanied by specific transcriptomic alterations. Mol Plant Microbe Interact 23:651–664

Gaude N, Bortfeld S, Duensing N, Lohse M, Krajinski F (2012) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. Plant J 69:510–528

Gavito ME, Curtis PS, Mikkelson TN, Jakobsen I (2000) Atmospheric CO_2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. J Exp Bot 51:1931–1938

Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, Blancaflor EB, Udvardi MK, Harrison MJ (2009) *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. BMC Plant Biol 9:10

Goss MJ, de Varennes A (2002) Soil disturbance reduces the efficacy of mycorrhizal associations for early soybean growth and N_2 fixation. Soil Biol Biochem 34:1167–1173

Govindarajulu M, Pfeffer PE, Hairu J, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Gutjahr C, Novero M, Welham T, Wang T, Bonfante P (2011) Root starch accumulation in response to arbuscular mycorrhizal colonization differs among *Lotus japonicus* starch mutants. Planta 234:639–646

Harris D, Paul EA (1987) Carbon requirements of vesicular-arbuscular mycorrhizae. In: Safir GR (ed) Ecophysiology of VA mycorrhizal plants. CRC, Boca Raton, FL, pp 93–105

Harris D, Pacovsky RS, Paul EA (1985) Carbon economy of soybean-*Rhizobium-Glomus* associations. New Phytol 101:427–440

Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413–2429

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp. is crucial for the symbiotic relationship with plants. Plant Cell 23:3812–3823

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83

Jia Y, Gray VM, Straker CJ (2004) The influence of Rhizobium and arbuscular mycorrhizal fungi on Nitrogen and Phosphorous accumulation by *Vicia faba*. Ann Bot Lond 94:251–258

Jones MD, Durall DM, Tinker PB (1991) Fluxes of carbon and phosphorous between symbionts in willow ectomycorrhizas and their changes with time. New Phytol 119:99–106

Kawai Y, Yamamoto Y (1986) Increase in the formation and nitrogen fixation of soybean nodules by vesicular-arbuscular mycorrhiza. Plant Cell Physiol 27:399–405

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bucking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882

Koch KE, Johnson CR (1984) Photosynthate partitioning in slit root Citrus seedlings with mycorrhizal root systems. Plant Physiol 75:26–30

Koide R (1985) The nature of growth depressions in sunflower caused by vesicular arbuscular mycorrhizal infection. New Phytol 99:449–462

Koide R, Elliott G (1989) Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis. Funct Ecol 3:252–255

Krajinski F, Hause B, Gianinazzi-Pearson V, Franken P (2002) Mtha1, a plasma membrane H + -ATPase gene from *Medicago truncatula*, shows arbuscule-specific induced expression in mycorrhizal tissue. Plant Biol 4:754–761

Kucey RMN, Paul EA (1981) Carbon flow in plant microbial associations. Science 213:473–474

Kucey RMN, Paul EA (1982) Carbon flow, photosynthesis and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). Soil Biol Biochem 14:407–412

Lodwig E, Poole P (2003) Metabolism of Rhizobium bacteroids. Crit Rev Plant Sci 22:37–78

Luis I, Lim G (1988) Differential response in growth and mycorrhizal colonisation of soybean to inoculation with two isolates of *Glomus clarum* in soils of different P availability. Plant Soil 112:37–43

Maekawa S, Sato T, Asada Y, Yasuda S, Yoshida M, Chiba Y, Yamaguchi J (2012) The Arabidopsis ubiquitin ligases ATL31 and ATL6 control the defense response as well as the carbon/nitrogen response. Plant Mol Biol 79:217–227

Maillet F, Poinsot V, Andre' O, Puech-Pages V, Haouy A, Guenier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Becard G, Denarie J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 649:58–63

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 59:89–102

Mortimer PE, Archer E, Valentine AJ (2005) Mycorrhizal C costs and nutritional benefits in developing grapevines. Mycorrhiza 15:159–165

Mortimer PE, Pérez-Fernández MA, Valentine AJ (2008) The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biol Biochem 40:1019–1027

Mortimer PE, Perez-Fernandez MA, Valentine AJ (2009) NH_4^+ nutrition affects the photosynthetic and respiratory C sinks in the dual symbiosis of a mycorrhizal legume. Soil Biol Biochem 41:2115–2121

Mortimer PE, Perez-Fernandez MA, Valentine AJ (2012) Arbuscular mycorrhiza maintains nodule function during external NH_4^+ supply in *Phaseolus vulgaris* (L.). Mycorrhiza 22:237–245

Motosugi H, Yamamoto Y, Naruo T, Kitabyashi H, Ishi T (2002) Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with an arbuscular mycorrhizal fungus *Gigaspora margarita*. Vitis 41:21–25

Murphy PM (1986) Effect of light and atmospheric carbon dioxide concentration on nitrogen fixation by herbage legumes. Plant Soil 95:399–409

Nielson KL, Amram E, Lynch JP (2001) The effect of phosphorous availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. J Exp Bot 52:329–339

Nwoko H, Sanginga N (1999) Dependence of promiscuous soybean and herbaceous legumes on arbuscular mycorrhizal fungi and their response to bradyrhizobial inoculation in low P soils. Appl Soil Ecol 13:251–258

Olivera M, Tejera N, Iribarne C, Ocana A, Lluch C (2004) Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorous. Physiol Plantarum 121:498–505

Olsson PA, Johansen A (2000) Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol Res 104:429–434

Orcutt DM, Nilsen ET (2000) The physiology of plants under stress. Soil and biotic factors. Wiley, New York, NY

Othman WMW, Li TA, Tmannetje L, Wassink GY (1991) Low-level phosphorus supply affecting nodulation, N_2 fixation and growth of Cowpea (*Vigna unguiculata* L. Walp). Plant Soil 135:67–74

Pacovsky RS, Fuller G, Stafford AE, Paul EA (1986) Nutrient and growth interactions in soybeans colonized with *Glomus fasciculatum* and *Rhizobium japonicum*. Plant Soil 92:37–45

Parrent JL, Vilgalys R (2009) Expression of genes involved in symbiotic carbon and nitrogen transport in *Pinus taeda* mycorrhizal roots exposed to CO_2 enrichment and nitrogen fertilization. Mycorrhiza 19:469–479

Pearson JN, Jakobsen I (1993) The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with 32P and 33P. New Phytol 124:489–494

Pearson JN, Schweiger P (1993) *Scutellospora calospora* (Nicol. & Gerd) associated with subterranean clover: dynamics of soluble carbohydrates. New Phytol 124:215–219

Peng S, Eissenstat DM, Graham JH, Williams K, Hodge NC (1993) Growth depression in mycorrhizal citrus at high-phosphorous supply. Plant Physiol 101:1063–1071

Provorov NA, Tikhonovich IA (2003) Genetic resources for improving nitrogen fixation in legume-rhizobia symbioses. Genet Resour Crop Evol 359:907–918

Rodriguez JA, Morcillo RL, Vierheilig H, Ocampo JA, Ludwig-Müller J, Garrido JM (2010) Mycorrhization of the notabilis and sitiens tomato mutants in relation to abscisic acid and ethylene contents. J Plant Physiol 167:606–613

Sa TM, Israel DW (1991) Energy status and function of phosphorous-deficient soybean nodules. Plant Physiol 97:928–935

Sanders FE, Tinker PB (1971) Mechanism of absorption of phosphate from soil by Endogone mycorrhizae. Nature 233:278–279

Schortemeyer J, Atkin OK, McFarlane N, Evans JR (1999) The impact of elevated atmospheric CO2 and nitrate supply on growth, biomass allocation, nitrogen partitioning and N2 fixation of *Acacia melanoxylon*. Aust J Plant Physiol 26:737–747

Selosse MA, Rousset F (2011) The plant-fungal marketplace. Science 333:828–829

Sieverding E (1989) Ecology of VAM fungi in tropical agrosystems. Agr Ecosyst Environ 29:369–390

Smith SE (1980) Mycorrhizas of autotrophic higher plants. Biol Rev 55:475–510

Smith SE (1982) Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. New Phytol 90:293–303

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol 92:75–87

Sussanna JF, Hartwig UA (1996) The effect of elevated CO2 on symbiotic nitrogen fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. Plant Soil 187:321–332

Tejeda-Sartorius M, Martínez de la Vega O, Délano-Frier JP (2008) Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning. Physiol Plant 133:339–353

Thompson BD, Robson AD, Abbott LK (1990) Mycorrhizas formed by *Gigaspora calospora* and *Glomus fasciculatum* on subterranean clover in relation to soluble carbohydrate concentrations in roots. New Phytol 114:405–411

Thorneley RNF (1992) Nitrogen fixation-new light on nitrogenase. Nature 360:532–533

Toro M, Azcon R, Barea JM (1998) The use of isotopic dilution techniques to evaluate the interactive effects of Rhizobium genotype, mycorrhizal fungi, phosphate solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol 138:265–273

Toussaint JP, St-Arnaud M, Charest C (2004) Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* (Schenck & Smith) and Ri T-DNA roots of *Daucus carota* L. in an in vitro compartmented system. Can J Microbiol 50:251–260

Trépanier M, Bécard G, Moutoglis P, Willemot C, Gagné S, Avis TJ, Rioux JA (2005) Dependence of arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. Appl Environ Microbiol 71:5341–5347

Udvardi MK, Tabata S, Parniske M, Stougaard M (2005) *Lotus japonicus*: legume research in the fast lane. Trends Plant Sci 10:222–228

Vadez V, Beck DP, Lasso JH, Drevon J-J (1997) Utilization of the acetylene reduction assay to screen for tolerance of symbiotic N2 fixation to limiting P nutrition in common bean. Physiol Plantarum 99:227–232

Valentine AJ, Kleinert A (2007) Respiratory responses of arbuscular mycorrhizal roots to short-term alleviation of P deficiency. Mycorrhiza 17:137–143

Vance CP (2002) Root-bacteria interactions: symbiotic nitrogen fixation. In: Waisel Y, Eschel A, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Dekker, New York, NY, pp 839–867

Vesjsadova H, Siblikova D, Gryndler M, Simon T, Miksik I (1993) Influence of inoculation with *Bradyrhizobium japonicum* and *Glomus claroideum* on seed yield of soybean under greenhouse and field conditions. J Plant Nutr 16:619–629

Vessey JK, Layzell DB (1987) Regulation of assimilate partitioning in soybean. Initial effects following change in nitrate supply. Plant Physiol 83:341–348

Williams K, Percival F, Merino J, Mooney HA (1987) Estimation of tissue construction cost from heat of combustion and organic nitrogen content. Plant Cell Environ 10:725–734

Yoneyama K, Xie X, Sekimoto H, Takeuchi Y, Ogasawara S, Akiyama K, Hayashi H, Yoneyama K (2008) Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. New Phytol 179:484–494

Zsögön A, Lambais MR, Benedito VA, Figueira AVO, Peres LEP (2008) Reduced arbuscular mycorrhizal colonization in tomato ethylene mutants. Sci Agric 65:259–267