

# Carbon transfer between plants and its control in networks of arbuscular mycorrhizas

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## Summary

1. Two studies using the stable-isotope  $^{13}\text{C}$  have shown that large amounts of carbon can move between plants linked by arbuscular mycorrhizal fungi. Quantities comparable to the carbon cost of the symbiosis for an individual plant may be transferred.
2. We measured C transfer between linked plants of the grass *Cynodon dactylon* ( $\text{C}_4$ ,  $\delta^{13}\text{C} \approx -14\text{‰}$ ) and the herb *Plantago lanceolata* ( $\text{C}_3$ ,  $\delta^{13}\text{C} \approx -28\text{‰}$ ). To test the hypothesis that the carbon transferred between plants remained in fungal structures at all times, plants were grown for two harvests: at the first harvest they were clipped to ground level, so that shoot re-growth required the transport of carbon from the roots. We also tested the influence of the direction of growth of the fungus, to determine whether C was transported out of or into a newly colonized root, and of growing plants in elevated  $\text{CO}_2$ , to increase the availability of carbon compounds in the roots.
3. Large amounts of C were transferred between linked plants, more so into *Plantago* than into *Cynodon* roots. Transfer occurred whether root systems were separated by a 20  $\mu\text{m}$  mesh, that excluded roots but not hyphae, or a 0.45  $\mu\text{m}$  mesh, intended to act as a barrier to hyphae as well. We believe that the high root densities achieved in the experiment allowed hyphae to cross the finer mesh between the two dense root mats.
4. Clipping the plants did not result in any movement of C from roots to shoots, thus confirming the prediction that all C transferred remains in fungal structures.
5. The direction of growth of the fungus did not affect the direction of transfer, nor did the  $\text{CO}_2$  concentration in which the plants were grown.
6. The amount of C transferred was a positive correlate of the frequency of vesicles in the roots but a negative correlate of the frequency of hyphae. If C were moving into developing colonization units, thus effectively giving the plant a 'free' symbiosis, the correlation with internal hyphae should be positive. The positive correlation with vesicles suggests that C is moving into fungal storage structures.
7. We propose a myco-centric view of the phenomenon of interplant C transfer, in which the fungal colonies within roots are seen as parts of an extended mycelium between which the fungus moves resources depending on the dynamics of its own growth. We do not believe that the transfer has an impact on plant C budgets or fitness, but that it may be a major element in the understanding of fungal C budgets.

*Key-words:* arbuscular mycorrhiza,  $\text{C}_4$ ,  $\text{C}_3$ , carbon transport, stable isotopes,  $^{13}\text{C}$

*Functional Ecology* (1998) 12, 406–412

## Introduction

We have recently quantified the movement of carbon between plants linked by a common arbuscular mycorrhizal network, both by using the natural difference in discrimination against the stable-isotope  $^{13}\text{C}$  found in  $\text{C}_3$  and  $\text{C}_4$  plants (Watkins *et al.* 1996), and by artificially altering the  $^{13}\text{C}$  content of half the plants in a community of  $\text{C}_3$  plants (Graves *et al.* 1997). Although it has been known for some time that

carbon can move between mycorrhizally linked plants (Hirrel & Gerdemann 1979, Francis & Read 1984), previous workers had used the radioactive isotope  $^{14}\text{C}$  and had been unable to measure the amounts of carbon moving because the specific activity of the source was unknown. As a result, the tantalizing situation developed where it was known that a phenomenon existed that could have profound ecological consequences, such as altering competitive balances

or improving seedling survival, but the data to determine whether or not the phenomenon was sufficient to produce these results were unavailable (Newman 1988).

Stable isotopes overcome these problems because the  $^{13}\text{C}$  concentration (typically expressed as  $\delta^{13}\text{C}$  in ‰) can easily be determined for both (or all) plants in a network and the amounts of carbon that have moved between plants calculated accurately. We found that typically between 0 and 10% of the carbon in the roots of a plant was derived from another, linked plant, with occasional examples being found of values as high as 40%. Estimates of the carbon cost of mycorrhizal colonization are typically around 10% (e.g. Snellgrove *et al.* 1982; Koch & Johnson 1984); the results from stable-isotope analyses therefore suggested that the entire carbon demand of a developing colonization unit could be supplied from another plant. If this is true, then it invalidates cost-benefit analyses of the mycorrhizal symbiosis, which have always assumed that the cost of colonization is the carbon required to construct and maintain the fungus in and associated with the root (Fitter 1991; Koide 1991). However, we could find no evidence that carbon transferred between plants was transported from the roots to the shoots, which implied that the carbon remained in fungal structures and that the direction of the fluxes across the plant-fungus interface (C from plant to fungus, P from fungus to plant) was unaffected.

A major obstacle to understanding the phenomenon of interplant transfer is that it is not known whether the carbon remains in fungal structures at all times and at what level the transfer is controlled. We therefore undertook an experiment similar to that of Watkins *et al.* (1996) using the natural difference in  $\delta^{13}\text{C}$  between linked plants of the grass *Cynodon dactylon* ( $\text{C}_4$ ,  $\delta^{13}\text{C} \approx -14\text{‰}$ ) and the herb *Plantago lanceolata* ( $\text{C}_3$ ,  $\delta^{13}\text{C} \approx -28\text{‰}$ ) to trace C movement in order to test unequivocally the hypothesis that transferred carbon remains in the fungus. In addition, we wished to test a second hypothesis, namely that the direction of colonization would affect the direction of transfer, either because the fungus cannot or does not obtain C from the newly colonized plant, or because the fungus obtains more than it needs for the growth of the developing colonization unit and transfers the excess to other parts of the mycelium. Finally, we included a treatment at elevated  $\text{CO}_2$ , in an attempt to manipulate available carbon levels in the roots of colonized plants: the hypothesis here was that plants grown at elevated  $\text{CO}_2$  would be likely to act as better C sources than those grown at ambient  $\text{CO}_2$  concentrations.

### Materials and methods

Twenty-eight Perspex boxes (23 cm  $\times$  23 cm  $\times$  10 cm high) were divided into four equal quadrants by two dividers, comprising nylon mesh held on a Perspex

frame. In 24 of the boxes one of the dividers was of 20  $\mu\text{m}$  mesh (Stanlar P/N $^\circ$ 25T11-20) and the other of 0.45  $\mu\text{m}$  mesh (Sartorius 25006-41BL); the former was designed to act as a barrier to roots but not hyphae, the latter to both roots and hyphae but not to water movement. In the remaining four boxes, both meshes were of 0.45  $\mu\text{m}$  mesh. Only four replicates of 0.45  $\mu\text{m}$   $\times$  0.45  $\mu\text{m}$  mesh were used because we expected very low variability in the  $\delta^{13}\text{C}$  values of these plants. The boxes were filled with a sandy loam soil. In each box there were two plants of the  $\text{C}_4$  grass *C. dactylon* on one side and two of the  $\text{C}_3$  herb *P. lanceolata* on the other, one in each quadrant (see Watkins *et al.* 1996 for details of source and propagation). Twenty boxes were placed in a glasshouse at ambient  $\text{CO}_2$  concentration ( $\approx 380 \pm 30 \mu\text{l l}^{-1}$ ; target value and range) and eight boxes were placed in a separate compartment of the same glasshouse maintained at  $610 \pm 30 \mu\text{l l}^{-1}$ . Plants and  $\text{CO}_2$  fumigation control were exchanged between the compartments every week to remove chamber effects. The air in the  $\text{CO}_2$  fumigated chamber had a more negative  $\delta^{13}\text{C}$  value owing to the addition of bottled gas ( $\delta^{13}\text{C} \approx -30\text{‰}$ ). This resulted in both species having considerably lower  $\delta^{13}\text{C}$  values in the high  $\text{CO}_2$  treatment although the difference in  $^{13}\text{C}$  discrimination between the two was maintained.

There were three treatments intended to vary the nature of the linkages between plants. In eight of the boxes at ambient  $\text{CO}_2$  (and the eight held at elevated  $\text{CO}_2$ ), the meshes were arranged so that the 20  $\mu\text{m}$  mesh ran between the *Cynodon* and the *Plantago*, and that plants of the same species were separated by the 0.45  $\mu\text{m}$  mesh; this treatment is referred to as 'interspecifically linked', because mycorrhizal links could form between *Cynodon* and *Plantago* and not between pairs of the same species. In another eight boxes at ambient  $\text{CO}_2$ , the meshes were arranged so that the 0.45  $\mu\text{m}$  mesh lay between *Cynodon* and *Plantago* plants; this was intended to prevent the formation of interspecific mycorrhizal links, but to permit intraspecific links. This treatment is called 'intraspecifically linked'. The four boxes with 0.45  $\mu\text{m}$  mesh between all pairs of plants acted as controls.

We initiated mycorrhizal colonization by adding inoculum (roots of *Plantago lanceolata* colonized by *Glomus mosseae*, UY21) to only one plant of each species in each box; the other was colonized by hyphae crossing the 20  $\mu\text{m}$  mesh from another plant. In the interspecifically linked boxes this other plant was of a different species, whereas in the intraspecifically linked boxes it was of the same species. Our hypothesis here was that transport would be determined by the direction of hyphal growth, although we could not distinguish a priori between the possibility that carbon would move in the direction of growth (i.e. the colonized plant receives mycorrhizal carbon) or in the opposite direction (the colonized plant is effectively parasitized by the fungus). If direction of

colonization affects C transfer, we predicted that the roots of the two plants of the same species in interspecifically linked boxes would have different  $\delta^{13}\text{C}$  values. It is possible, however, that direction of colonization directly affects  $\delta^{13}\text{C}$ , perhaps by altering the fractionation of C compounds in roots; in that case, we predicted that the two plants in the intraspecifically linked boxes would also have different  $\delta^{13}\text{C}$ .

Plants were put into boxes on 26 May 1995. The glasshouse was unheated and lit by natural daylight. Shoots of all plants were cut on 31 August 1995 and then allowed to re-grow, before a complete harvest on 21 September 1995. Root samples on each occasion were obtained from 2 cm  $\times$  2 cm  $\times$  10 cm high cores in each of the four compartments of each box, at a distance of 2 cm from the mesh. Roots were carefully washed from the soil and subsamples used for stable-isotope analysis and for mycorrhizal scoring.

Plant samples were dried at 70 °C for 48 h, and then finely ground using a Retsch Mixer ball mill. After re-drying at 70 °C for 24 h, 1 mg samples were weighed in duplicate into 6 mm  $\times$  4 mm tin cups for stable-isotope analysis by continuous flow isotope ratio mass spectrometry, using a Europa Scientific ANCA-NT Stable Isotope Analyser with ANCA-NT Solid/Liquid Preparation Module (Europa Scientific Ltd, Crewe, UK). Simultaneous measurements of  $^{13}\text{C}$ , %C and %N were made, with a precision of  $< 0.2\%$ . Standards were 1 mg leucine-citric acid mixture. Results for  $\delta^{13}\text{C}$  are expressed relative to the PDB standard in ‰.

Carbon transfer between plants would have occurred if the  $\delta^{13}\text{C}$  value of the roots of an interspecifically linked plant was nearer that of its interspecific partner than expected (i.e. less negative in *P. lanceolata* and more negative in *C. dactylon*). However, there is always natural variation among plants in  $\delta^{13}\text{C}$ . Carbon transfer can therefore be quantified by plotting the difference between root and shoot  $\delta^{13}\text{C}$  against root  $\delta^{13}\text{C}$ . This removes the effect of interplant differences owing to, for example, different stomatal conductances. A slope of unity in this plot implies that all the variation in root  $\delta^{13}\text{C}$  is owing to import of carbon from a source other than the shoots, rather than natural interplant differences, as explained by Watkins *et al.* (1996). Amounts transferred can then be estimated from the difference between root and shoot  $\delta^{13}\text{C}$ .

Roots were stained with acid fuchsin to reveal mycorrhizal colonization by epifluorescence (Merryweather & Fitter 1991). Frequency of colonization of arbuscules, hyphae and vesicles was estimated following McGonigle *et al.* (1990).

## Results

### EXTENT OF TRANSFER

In all four treatments at ambient  $\text{CO}_2$ , the difference between root and shoot  $\delta^{13}\text{C}$  signals was linearly related to the root  $\delta^{13}\text{C}$  signal (Fig. 1) and the relationship was the same at the two harvests, except for a

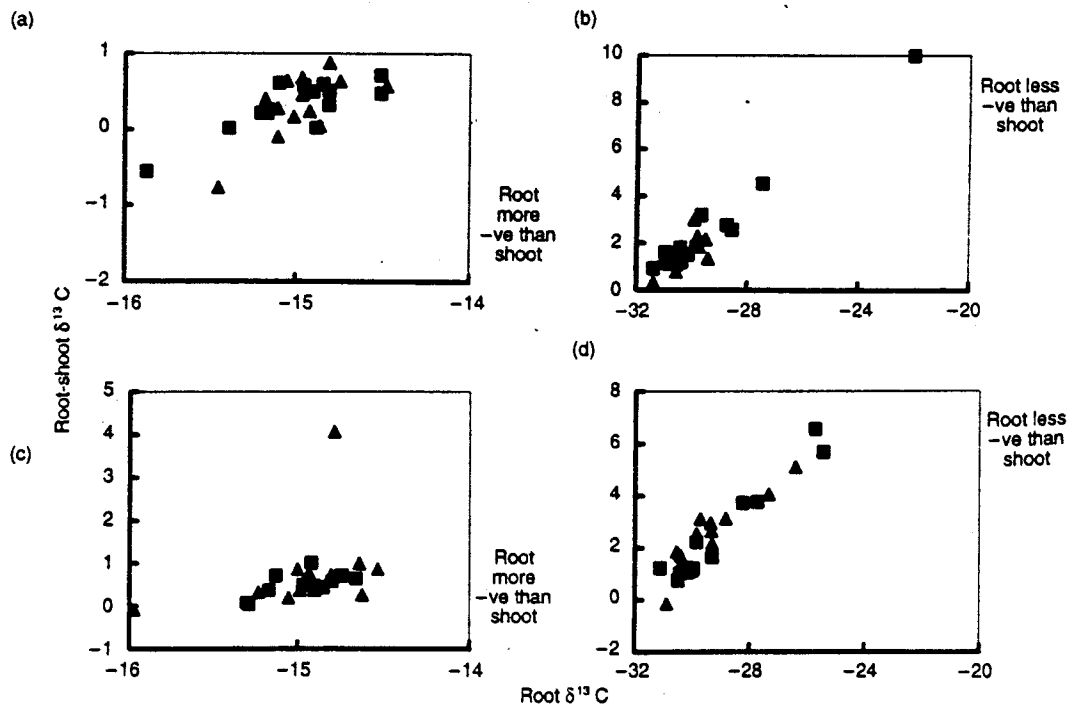


Fig. 1. The relationship between the difference in root and shoot  $\delta^{13}\text{C}$  values and root  $\delta^{13}\text{C}$  for (a,b) interspecifically linked and (c,d) intraspecifically linked plants of *Cynodon dactylon* (a,c) and *Plantago lanceolata* (b,d), all grown at  $380 \mu\text{l l}^{-1} \text{CO}_2$ . A slope of unity in this plot implies that all variation in the root  $\delta^{13}\text{C}$  value is a result of import of differently labelled carbon, rather than to natural fluctuations in  $\delta^{13}\text{C}$  of both root and shoot. Regression coefficients (with  $R^2$  values in parenthesis) are: (a) 0.961 (0.650), (b) 0.968 (0.924), (c) 0.671 (0.427) and (d) 0.986 (0.885). The value for (c) omits the obvious outlier, -ve. negative. Data are from the two harvests: ■ harvest 1; ▲ harvest 2.

single pronounced outlier in the intraspecifically linked *Cynodon* at harvest 2. In three of the four treatments, the slope of the regression was almost exactly unity (0.96–0.99) and in the fourth the value was 0.67, but was not significantly different from 1 (95% confidence limits were 0.336 and 1.006). Amounts of carbon transferred varied widely within a treatment but were generally much greater in *P. lanceolata* than in *C. dactylon*: differences in  $\delta^{13}\text{C}$  ranged from zero to 10 in *P. lanceolata* (although most were < 4) but, with a single exception, they were < 1 in *C. dactylon*. A root–shoot difference of 4 in  $\delta^{13}\text{C}$  implies that 12.5% (4/32) of the carbon in *P. lanceolata* roots was derived from *C. dactylon*, if the control value is taken as –32‰ (Fig. 1); however, the exact transfer values could not be calculated because the 0.45  $\mu\text{m}$  mesh failed, in this experiment, to prevent transfer. Root–shoot  $\delta^{13}\text{C}$  differences were as great or greater in the ‘intraspecifically linked’ treatment (in which interspecific carbon transfer should have been prevented by the fine mesh) as in the interspecifically linked treatment and transfer also occurred in the controls.

#### DIRECTION OF TRANSFER

There was no evidence that the direction of colonization affected the transfer. This was tested by comparing the root–shoot difference in  $\delta^{13}\text{C}$  in roots of donor and

receiver plants (i.e. those that were initially inoculated and those that became colonized from the inoculated plant). A paired *t*-test was used on the value of (root  $\delta^{13}\text{C}$  donor – shoot  $\delta^{13}\text{C}$  donor) – (root  $\delta^{13}\text{C}$  receiver – shoot  $\delta^{13}\text{C}$  receiver). If this value differs from zero, direction affects transfer: Table 1 shows that it did not.

#### FATE OF TRANSFERRED CARBON

Clipping the plants did not result in a change in the relative values of root and shoot  $\delta^{13}\text{C}$  between harvests 1 and 2. Data from harvest 2 followed exactly the same relationship as that from harvest 1 (Fig. 1). This demonstrates that the carbon that is transferred remains in fungal structures. Regression of shoot–root  $\delta^{13}\text{C}$ , as a correlate of C transfer, on the parameters of mycorrhizal colonization of roots reveals a consistent pattern in which transfer is lower where hyphal colonization is high and generally greater where vesicle colonization of roots is high (Table 2).

#### CONTROL OF TRANSFER: EFFECT OF ELEVATED CO<sub>2</sub>

Transfer was observed under elevated and ambient CO<sub>2</sub> (Fig. 2). For *C. dactylon* under elevated CO<sub>2</sub>, the slope of the regression of root–shoot  $\delta^{13}\text{C}$  on root  $\delta^{13}\text{C}$  was unity, with a single outlier, and for *P. lanceolata* it was lower but not significantly different from 1. Both results suggest that C transfer had occurred. In *C. dactylon* the values of  $\delta^{13}\text{C}$  were slightly larger than in ambient CO<sub>2</sub>, suggesting greater transfer, whereas in *P. lanceolata* they were much smaller. The data do not suggest that growing plants under elevated CO<sub>2</sub> concentrations alters the occurrence or extent of carbon transfer.

#### Discussion

A major surprise in this experiment was the failure of the 0.45  $\mu\text{m}$  mesh to prevent carbon moving between root systems. In a previous experiment (Watkins *et al.*

**Table 1.** Differences between root–shoot  $\delta^{13}\text{C}$  of donor and receiver plants in *Cynodon dactylon* and *Plantago lanceolata* at the two harvests. Data were only used for those boxes where all four plants provided material for analysis

Variable	<i>n</i>	Mean difference	SE	<i>t</i>	<i>P</i>
<i>P. lanceolata</i> : harvest 1	6	–2.43	1.29	1.89	0.12
<i>P. lanceolata</i> : harvest 2	6	0.12	0.49	0.25	0.81
<i>C. dactylon</i> : harvest 1	6	–0.03	0.37	0.09	0.93
<i>C. dactylon</i> : harvest 2	6	0.05	0.21	0.24	0.82

**Table 2.** Regression coefficients and *P*-values from a multiple regression of shoot–root  $\delta^{13}\text{C}$  (sign switched in *C. dactylon* so that positive coefficients indicate net transfer in both species) against percentage root length colonized by hyphae and vesicles as predictor variables. Coefficients in bold are significant at  $P < 0.05$ ; those in italic at  $0.05 < P < 0.10$

Treatment	Species and conditions	Hyphal colonization ( <i>P</i> -value)	Vesicle colonization ( <i>P</i> -value)	<i>P</i> value of the regression
Ambient CO <sub>2</sub>	interspecifically linked <i>P. lanceolata</i>	–0.022 (0.041)	<b>0.057</b> (0.011)	0.028
Ambient CO <sub>2</sub>	intraspecifically linked <i>P. lanceolata</i>	–0.078 (0.087)	–0.017 (0.755)	0.047
Enriched CO <sub>2</sub>	interspecifically linked <i>P. lanceolata</i>	–0.050 (0.067)	<b>0.085</b> (0.010)	0.017
Ambient CO <sub>2</sub>	interspecifically linked <i>C. dactylon</i>	–0.030 (0.011)	0.050 (0.173)	0.030
Enriched CO <sub>2</sub>	interspecifically linked <i>C. dactylon</i>	–0.023 (0.608)	0.042 (0.336)	0.605

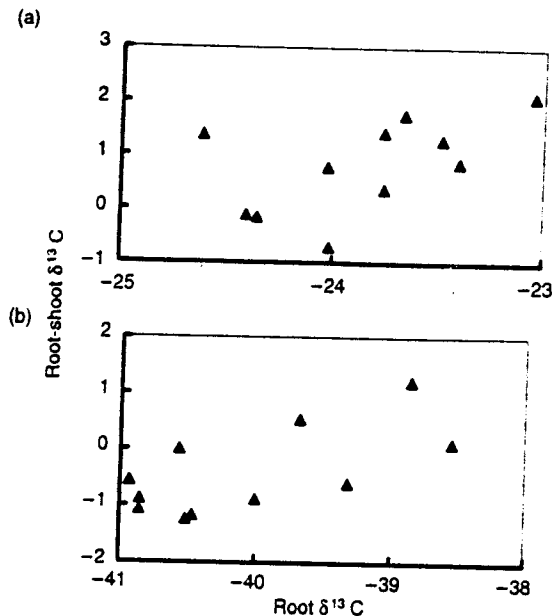


Fig. 2. The relationship between the difference in root and shoot  $\delta^{13}\text{C}$  values and root  $\delta^{13}\text{C}$  for interspecifically linked plants of (a) *Cynodon dactylon* and (b) *Plantago lanceolata*, grown at  $610 \mu\text{l l}^{-1} \text{CO}_2$ . The regression slopes are (a) 1.01 and (b) 0.631.

1996), we had found this barrier to be fully effective and it has been widely used by other researchers (e.g. Li, George & Marschner 1991; Jakobsen, Abbott & Robson 1992). The large root–shoot differences observed in the intraspecifically linked treatments were not a result of rupture of the mesh: meshes from all boxes were carefully inspected at the end of the experiment and in those few cases where the mesh was observed to have failed, the boxes were excluded from the analysis for both harvests. An alternative explanation is that carbon compounds diffused through the mesh but if this were the case, some of the carbon would have been taken up directly by the roots (Jones & Darrah 1995) rather than by the fungus and would be expected to have appeared in the shoots, at least at the second harvest: the data, however, clearly show that the same pattern of variable root and invariant shoot signal (i.e. slope of unity in Fig. 1) was observed at both harvests. Two explanations remain: first that carbon compounds are transferred across the mesh, taken up by the roots, but then remain fixed in the roots and do not mix with the general carbohydrate pool, and second that the mesh failed to act as a barrier because the root densities achieved in this experiment were much higher than in the previous study: at the final harvest dense root mats were found against the mesh. It appears more likely that hyphal links were able to form between roots against this mesh: the hyphae under these circumstances must have penetrated the  $0.45 \mu\text{m}$  mesh. If this explanation is correct, it has serious implications for other studies using meshes to control links: it may be necessary to adopt more stringent barriers, such as double meshes separated by narrow gaps.

A consequence of this unexpected result we were unable to quantify amounts of C transferred as we had done earlier (Watkins *et al.* 1996; *al.* 1997). Nevertheless, differences between shoot  $\delta^{13}\text{C}$  were similar to or larger than observed previously, confirming that large amounts of carbon (probably around 5–15% of total root carbon in many cases) were transferred.

One of the objectives in this study was to determine whether the plant ‘receivers’ of fungally transferred carbon could control or influence the transfer. Previous work has suggested that shading receiver plants increases the quantity transferred (Francis & Read 1984) and that clipping shoots reduces the ‘strength’ of the receiver (Waters & Borowicz 1990). The availability of carbon substrates in the root system has an influence on transfer, we would have expected to see an effect of growing plants in elevated  $\text{CO}_2$ . This treatment had no detectable effect, except possibly in reducing the extent of transfer in *P. lanceolata*. Elevating the concentration of  $\text{CO}_2$  does not always increase substrate supply especially in  $\text{C}_4$  species. However, previous work on the closely related species *Plantago major* has shown that under elevated  $\text{CO}_2$  growth, photosynthesis and root respiration are stimulated (Poorter, Pot & Lambers 1988).

The concept of plant control implies that plants may not have access to the transferred carbon, although it has been suggested that even if the carbon stays in the fungus it would be beneficial to the plant, because this would reduce the demand by the fungus on carbon from its immediate host (Newman 1988). The evidence that carbon moves from fungus to host is slight, however. Although Read, Francis & Finlay (1985) did find low levels of radioactivity in shoots of receiver plants in an experiment in which donors were fed with  $^{14}\text{CO}_2$ , the sensitivity of assays for radioactivity and the inability to determine the specific activity of the carbon pools in such experiments, mean that it is possible that the label represented carbon respired from the roots and re-fixed by the shoots. In Read *et al.*'s (1985) experiments, the radioactivity in shoots of mycorrhizal plants was only 0.041% of that in the roots, suggesting that if transport to shoots occurred, it was at a very slow rate; strikingly, when the receiver plant was grown in half or full shade, the amount of radioactivity in the roots increased but the fraction in the shoots decreased, to 0.027% and 0.001%, respectively, precisely what would be expected if the  $^{14}\text{C}$  in shoots was re-fixed photosynthetically rather than transported from roots to shoots.

If carbon can cross the fungus–plant interface in the opposite direction in receivers to that in donors, then it should have been detected in the shoots of the clipped plants at the second harvest. The fact that no such movement occurred is powerful evidence that the transferred carbon remains in the fungus, which can clearly be seen to be the case in the autoradiographs of *Festuca* and *Plantago* roots of Read *et al.* (1985).

The generally accepted view of mycorrhizal carbon transfer would interpret these data as suggesting that the plant to which transfer is occurring is effectively receiving a free symbiosis. Normally, mycorrhizal colonization requires the plant to supply around 5–10% of photosynthate to the fungus (Snellgrove *et al.* 1982; Koch & Johnson 1984). Cost-benefit analyses of the symbiosis (e.g. Fitter 1991; Koide 1991) assume that this carbon expenditure can be set against the phosphorus gain. If, however, some plants acquire the symbiosis without this cost, the net benefit of the symbiosis will be much greater. Potentially, such plants should gain a competitive advantage. This view is consistent with much of the evidence we have obtained but it does not explain (1) why some plants appear to receive much greater amounts of transferred carbon than others (Fig. 1), (2) why growing plants in elevated CO<sub>2</sub> has so little effect on transfer and, critically, (3) why the transfer is a positive function of vesicular colonization but a negative function of hyphal colonization. Because the early stages of colonization largely involve hyphal development, and it has been suggested that carbon transfer occurs principally across the hyphal interface (Smith & Smith 1996), an explanation that assumed plants can obtain a 'free' symbiosis would predict a positive relationship between transfer and hyphal colonization, because the carbon used to construct the developing hyphae in the root would be coming from a different plant.

We propose a radically different view, which is myco-centric, as opposed to the phytocentric one outlined above. We view the transfer as a fungal phenomenon and the roots that the extensive fungal mycelium colonizes as habitat patches for the fungus. As colonization units mature, the pattern of fungal growth changes from one dominated by hyphae and arbuscules to one dominated by vesicles, typically regarded as storage or reproductive structures. The positive correlation between transfer and vesicle development suggests that the fungus may acquire carbon from roots in which it has young infection units, predominantly hyphae and arbuscules, and transport it to older units, sometimes in other roots, where vesicles are developing. This hypothesis also explains why the pattern of transfer varies so much from plant to plant.

The phenomenon of mycorrhizal transfer of carbon may therefore be a reflection of the dispersed nature of the fungal mycelium. As such it is of great significance in understanding the ecology of the mycorrhizal fungus but probably of lesser importance to plant ecology, because the transfer does not alter plant carbon budgets or affect plant fitness directly. Traditionally, mycorrhizal researchers have concentrated on the impact of the symbiosis on plant performance. At best, attention to the fungus has been focused on spores (usually for taxonomic reasons) and on the internal structures in the root, because these could be seen to have a direct relationship to plant

behaviour. The external mycelium has been largely neglected (Sylvia 1990) but an understanding of the functioning of the symbiosis under field conditions requires that we are able to describe and predict the behaviour of the whole fungus. Physiological and ecological studies of the carbon budgets of whole mycelia and plants simultaneously are now required: stable-isotope studies will be a critical tool in those studies.

### Acknowledgements

This project was funded by the Natural Environment Research Council under the TIGER programme (GST/02/0645). We thank W. Stein for the isotope analyses. The Scottish Crop Research Institute receives grant-in-aid from the Scottish Office Agriculture, Environment and Fisheries Department.

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Received 28 February 1997; revised 28 July 1997; accepted 7 August 1997