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The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network

David Robinson[1,3] **and Alastair Fitter**[2]

[1] Cellular and Environmental Physiology Department, Scottish Crop Research Institute, Dundee DD2 5DA, UK
[2] Department of Biology, University of York, PO Box 373, York YO10 5YW, UK

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Abstract

Various claims have been made about the ecological significance of plant-to-plant carbon movement through common mycorrhizal networks (CMNs). Most suggest that resource competition among interconnected plants should be less important than previously thought. If true, that would profoundly alter our perception of how plants interact among themselves and with their environment. However, there are difficulties in quantifying the amounts of resource transferred via CMNs, ensuring that transfer is genuinely through hyphae, not soil, and understanding its control. Carbon movement has not been quantified in many of the published studies. Where it has, its likely functional role has not been clarified. Some recent, well-publicized research suggests that carbon transferred to trees via an ectomycorrhizal (EcM) network may be physiologically and ecologically important. Our view, however, is that the evidence for this remains equivocal. Appropriate controls for the possibility of carbon transfer via soil were not used under field conditions. In laboratory experiments, controls failed to clarify the role of EcM links in carbon transfer. To resolve some areas of uncertainty, abundances of ^{13}C have been measured to estimate carbon transfers via an arbuscular mycorrhizal (AM) network connecting grasses and forbs of the same or different species. Permeable barriers to roots and hyphae allowed any direct carbon transfer via soil to be detected. Large amounts of carbon (typically 10% of that in roots) were transferred between linked plants via the CMN. Transferred carbon was never transported into shoots of 'receiver' plants. It remained in roots, probably inside fungal structures and, therefore, unavailable to the plants into which it was apparently transferred. Carbon transfer via an AM network does not allow 'resource sharing' among linked plants. It is probably irrelevant to the botanical components of a community, but it may be fundamental for fungal members. The 'mycocentric' view is that fungal structures within roots are parts of extended mycelia through which fungi move carbon according to their own carbon demands, not those of their autotrophic hosts.

Key words: Arbuscular mycorrhiza, carbon, common mycorrhizal network, ectomycorrhiza.

Introduction

The 7 August 1997 issue of *Nature* was headlined 'The wood-wide web', announcing the publication of the latest report (Simard *et al.*, 1997*a*) of plant-to-plant carbon (C) transfer via a common mycorrhizal network (CMN). The paper itself, highlighted by Read's (1997) commentary, left *Nature* readers in no doubt that this intriguing phenomenon was important. Plants connected by a CMN could, it was claimed, share C.

This claim was not new. For example, Grime *et al.* (1987), suggested that grassland herbs connected by a CMN formed by arbuscular mycorrhizal (AM) fungi allowed C to be transferred from 'donor' to 'receiver' species in laboratory microcosms. But Simard *et al.* (1997*a*) were the first to suggest that the same was true for ectomycorrhizal (EcM) networks connecting different tree species in the field, and that the transfer of C was bidirectional. This, clearly, is big news. If plants in a community can, via a CMN, really share C, this would 'short-circuit' one of the main constraints to the acquisition of C by neighbouring plants, namely, competition. Interactions between neighbours would then be less of a

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[3] To whom correspondence should be addressed. Fax: +44 1382 562 426. E-mail: d.robinson@scri.sari.ac.uk

struggle for a meagre resource than a communal enterprise in which everyone (at least those in the CMN) got a share. That would change fundamentally our view of an important influence over the structure and dynamics of plant communities.

To determine whether plant-to-plant C transfer via a CMN can override normal competitive interactions between plants, four fundamental questions must be answered:

(1) How much C is transferred? If only tiny amounts are involved, the transfer is ecologically and physiologically trivial (assuming that the transferred C consists only of sugars, amino acids, etc., rather than hormones, nucleic acids, etc.).
(2) Does transferred C move into plant cells or remain in fungal structures? If it remains inside the fungus, it cannot be used by the plant as a C source and so will not alter the prevailing competitive relations with neighbouring plants, unless the transfer relieves the plant of the C cost of maintaining the symbiosis.
(3) Is transfer uni- or bidirectional? If C transfer can occur both to and from plants, it can genuinely be shared among them. If it can occur in only one direction, parasitism is possible.
(4) Are hyphal links involved, allowing control of transfer by the symbiosis? If C transfer occurs only or largely through soil, that would be seen as part of the normal C cycle and no special role for the CMN need be invoked. If it occurred only through the CMN, C transfer could, potentially, be influenced by any or all of the network's members (plant, as well as fungal).

Here, an attempt is made to answer these questions in the light of Simard *et al.*'s (1997*a*) findings, and also in relation to other studies which have made comparable claims for plant-to-plant C transfer, and to our own experimental observations. To start with, several fundamental biological and technical points about such work are noted.

Background: studying the occurrence and functioning of CMNs

The roots of most plants are colonized by mycorrhiza-forming fungi. Around two-thirds of plant species (Trappe, 1987) form arbuscular mycorrhizas with fungi in the Glomales, of which there are about 150 described species (Morton and Benny, 1990). One survey (Harley and Smith, 1983) listed 140 genera of angiosperms and gymnosperms in which ectomycorrhizal associations have been reported. The same survey listed 45 genera of basidiomycetes and 18 genera of ascomycetes in which at least one species forms ectomycorrhizas. It follows that colonization has little host-specificity. It is, therefore, highly probable that, in the field, roots of many different plant species will form a CMN with either AM or EcM fungi. Organic and inorganic solutes might then move, via the CMN, from plant to plant.

CMNs have been studied experimentally for many years (Newman, 1988). The technical problems in demonstrating unequivocally that plant-to-plant C transfer occurs via a CMN are formidable.

The *possibility* of plant-to-plant C transfer via both EcM and AM networks was demonstrated by many workers who administered $^{14}CO_2$ to the leaves of a 'donor' plant and measured the ^{14}C label in another to which it was connected by a CMN (see the review by Newman, 1988). Usually, most ^{14}C appeared in the roots of 'receiver' plants; much less in their leaves. Unconnected controls showed negligible increases in ^{14}C activity following labelling, but isotopic transfer alone cannot demonstrate a *net* elemental transfer. Isotope exchange may occur even if the amounts of element involved are negligible. If net C transfer can be demonstrated, there is still the problem of quantifying it. Unless the specific activity of the source $^{14}CO_2$ is known, and it rarely is, the radioactivity of a sink tissue cannot be converted into an amount of C. Without knowing how much C is transferred, it is impossible to say anything meaningful about the ecological importance of the phenomenon.

If a net C transfer can be measured, it must be shown to have occurred via the fungal links, and not some other route. For example, the loss into soil of C from the roots of one plant followed by its incorporation by roots or hyphae connected to another would give the illusion of plant-to-plant C transfer via a CMN. Such effects must be controlled. The standard way of doing this is to separate the root systems of plants by mesh barriers. A small mesh (e.g. 0.45 μm) allows only soil solution to pass, roots and (normally) hyphae being too wide to penetrate the barrier. A larger mesh (e.g. 20 μm) allows hyphae and soil solution to pass freely, and only roots are prevented from crossing the barrier.

'The wood-wide web': C transfer in ectomycorrhizal networks

Simard *et al.*'s (1997*a*) innovation was to dual-label (^{13}C and ^{14}C) the shoots of two tree species, *Betula papyrifera* and *Pseudotsuga menziesii*, whose roots were connected by an EcM network in the field. The labelling was done reciprocally, i.e. ^{13}C-labelled *B. papyrifera* connected to ^{14}C-labelled *P. menziesii* or ^{14}C-labelled *B. papyrifera* connected to ^{13}C-labelled *P. menziesii*. However, full reciprocity was possible only in the first of the two years' data they quoted. Plants were labelled for 2 h and harvested 9 d later for isotopic and growth analyses. $^{14}CO_2$ was supplied at near-ambient concentrations (*c.* 0.03%), but the higher detection threshold for ^{13}C meant that the

$^{13}CO_2$ concentration had to be above ambient, probably $>0.2\%$. The CO_2 concentrations needed for ^{13}C labelling alter the patterns of primary C assimilation or allocation which occur at ambient CO_2, as indicated by the different distributions of ^{13}C and ^{14}C in *B. papyrifera* and *P. menziesii* which Simard *et al.* (1997*b*) measured in a laboratory study on these species. It may, therefore, be unsafe to compare C fluxes based on ^{13}C and ^{14}C labelling done at different source concentrations. This point requires further investigation.

In Simard *et al.*'s (1997*a*) experiment, transferred C moved from root to shoot, in marked contrast to AM networks (see below). Up to 13% of transferred C was found in shoots of *P. menziesii* and up to 45% in *B. papyrifera.* If these very large fractions are found to be general, it seems that in EcM systems there is, therefore, the potential for C transfer through the CMN to make a genuine difference to plants' C requirements.

There is some evidence, however, that C transfers to the shoots of EcM-connected plants may not always be so large. Read *et al.* (1985) exposed *Pinus contorta* to $^{14}CO_2$ when plants were linked by one of two *Suillus* species. ^{14}C transfer to shoots averaged only 9% of that in roots (see their Table 1). Transfer to the shoots of plants connected by *S. granulatus* were not significantly different from background (as measured in non-mycorrhizal controls), suggesting that an EcM linkage is no guarantee of enhanced C transfer.

Simard *et al.* (1997*a*) suggested that they showed both net and bidirectional transfer of C. However, transport of ^{14}C from *B. papyrifera* to *P. menziesii* (average 6.6% of total fixed C) was 10-fold greater than the reverse transfer of ^{13}C from *P. menziesii* to *B. papyrifera* (average 0.6%, calculated as bidirectional transport less twice net; their Table 1). The latter figure is very small and may well lie within the error limits of the calculations. Importantly, therefore, it appears that they may have shown genuine one-way (i.e. *not* bidirectional) transport in an EcM network, which has not yet been confirmed in AM systems. One-way transport, combined with fungus–plant transfer, opens the possibility of parasitism by one plant on another, via mycorrhizal links.

It was also suggested by Simard *et al.* (1997*a*) that the C transfer they observed was through hyphal links, because 'transfer to the arbuscular mycorrhizal *Thuja plicata* [in the same systems] was only [*sic*] 18% of the total transfer between *B. papyrifera* and *P. menziesii*'. This is a surprisingly large figure. It shows that significant quantities of C could be transferred between *unlinked* plants. An alternative explanation is that C was lost from EcM hyphae into the soil, either by death, exudation or secretion, and subsequently captured either by other hyphae or even by roots. Indeed, it seems likely that hyphal densities of the EcM fungi, with two hosts present, were greater than those of the AM fungi, especially at those locations in the soil where other hyphae of EcM fungi were losing C to the soil. This could explain the greater C transfer to the EcM than the AM plant and, possibly, also the root-to-shoot transfer.

The results presented by Simard *et al.* (1997*a*) are important, but they do not yet eliminate the possibility of plant-to-plant C transfer not involving EcM hyphal links under field conditions, because no physical barriers were used to prevent hyphal contact between plants; this is difficult to achieve, even in the laboratory (see Fitter *et al.* 1998). In Simard *et al.*'s (1997*b*) laboratory study, hyphae connecting *B. papyrifera* and *P. menziesii* were severed, but the wide variation among replicates meant that severing had no statistically significant effect on apparent C transfer. The role of EcM links in plant-to-plant C transfer remains uncertain even under laboratory conditions, and alternative explanations must be considered. One possible alternative is that if C transfer occurs because of foraging by fungal hyphae, then the plant which gains from the interaction will simply be that best able to acquire resources from the fungus, irrespective of links.

Until bidirectionality of plant-to-plant C transfer via an EcM network is demonstrated, the evidence so far suggests only that mycorrhizal links are a possible route for C transfer from one plant to another, i.e. they may allow parasitic rather than mutualistic associations among connected plants.

The apparent plant-to-plant transfer of C via an AM network differs in many ways from that in an EcM network, and it is these to which we now turn.

'The in-turf net': C transfer in arbuscular mycorrhizal networks

In their well-known 'microcosm' experiment, Grime *et al.* (1987) labelled shoots of the canopy dominant *Festuca ovina* with $^{14}CO_2$. After 72 h, they measured ^{14}C in shoots of other, less-abundant grasses and forbs growing with *F. ovina* in artificial turves. Most radioactivity was found in shoots of species whose roots were colonized by AM fungi. Less radioactivity was found in the roots of non-mycorrhizal species (e.g. *Rumex acetosa*). From this result, Grime *et al.* suggested AM networks could promote floristic diversity by allowing C transfer to occur from dominant plant species to subordinate ones in a CMN, but not to those unable to form AM associations. However, Grime *et al.* did not, in fact, demonstrate or quantify net C transfer (only that of the ^{14}C label), nor show that any transfer occurred via a CMN as opposed to alternative pathways (Bergelson and Crawley, 1988).

Read *et al.* (1985) measured ^{14}C transfer from labelled *Plantago lanceolata* and *Festuca ovina* linked by an AM network. The radioactivity in shoots of mycorrhizal *F. ovina* was only 0.041% of that in the roots (see their

Table 3). This suggests that any transport of C to shoots occurred very slowly. An alternative explanation is that some $^{14}CO_2$ respired by roots and microbes may have been re-fixed during photosynthesis, thus appearing in shoot material. As Fitter *et al.* (1998) noted, when Read *et al.*'s 'receiver' plants were grown in half or full shade, the amount of radioactivity in their roots increased, but the fraction in the shoots decreased, to 0.027% in half shade and 0.001% in full shade. This is precisely what would be expected if the ^{14}C in shoots had got there via photosynthesis rather than transport from roots to shoots.

Watkins *et al.* (1996) used natural abundances (δ) of ^{13}C to detect and quantify transfer of C from a C_3 plant connected by an AM network to a C_4 plant, and vice versa. They did not have to label a 'donor' plant with isotopically distinct CO_2, but relied instead on the difference in $\delta^{13}C$ among C_3 and C_4 plants when grown with the same CO_2 source, a difference which arises from their contrasting photosynthetic pathways and the extents to which these discriminate against ^{13}C. Moreover, the root systems were separated by meshes which did or did not allow hyphae to pass from plant to plant. The meshes controlled for the possibility that C transfer might occur through soil, rather than hyphae.

This approach allowed the gross, unidirectional transfer of C, via the CMN, from *Plantago lanceolata* (C_3) into *Cynodon dactylon* (C_4) to be estimated over 10 weeks. It averaged 10% of the total C in the roots of *C. dactylon*, but there was a large variation among replicates. It was not possible to measure net or bidirectional transfers with this technique because of an innate difference in $\delta^{13}C$ between roots and shoots of *P. lanceolata* masking any import of C from *C. dactylon*. However, 10% of root C is a large fraction, about the same as that estimated as the net C cost to a plant of a mycorrhizal symbiosis and, potentially, therefore, significant quantities of C may be transferred among root systems via an AM network.

However, using the same approach, Fitter *et al.* (1998) showed that this transferred C *remained in roots* (or, at least, within the limits of detection of the $\delta^{13}C$ technique). It was never transferred into shoots. That strongly suggests that transferred C is retained in fungal structures in the roots of 'receiver' plants. If it had not, some of the transferred C released into the root apoplast or symplast would inevitably have found its way into the vascular system and soon become detectable in shoots. This finding casts doubt on whether C transferred from plant-to-plant via an AM network is likely to have any physiological significance for the 'receiver'.

Conclusions

On the basis of the available evidence, it is not possible to conclude that plant-to-plant C transfer via a CMN has any significance for the composition or functioning of plant communities. Key experiments remain to be done, however. Chief among these is the need to test for genuine bidirectionality of C transfer in the absence of possible artefacts (see above) and with the presence of barriers capable of stopping the formation of hyphal links, and to verify that large amounts of transferred C may appear in shoots of 'receiver' plants connected by an EcM network.

The focus on CMNs has, so far, been exclusively phytocentric. The fungal mycelium is assumed to be a passive conduit for solute transfer between connected plants. Experiments have been done with the aim of finding out only what *plants* gain from being part of a CMN. We (Fitter *et al.*, 1998) have proposed an alternative, 'mycocentric', view and ask, instead, 'What's in it for the fungus?'

In AM networks, it may be that C is acquired by young infection units (e.g. hyphae and arbuscules) and then transferred to older, storage structures (e.g. vesicles) wherever they might be in a dispersed mycelium. Some of these will, inevitably, be in the roots of other plants. The result is that C is transferred from one plant to another, *but it remains as fungal C.* Plant-to-plant C transfer would then be largely under the control of the fungus, as dictated by its own C demands. Plants are then essentially habitat patches for the AM fungi, and not the grateful beneficiaries of non-Darwinian fungal altruism (Robinson, 1991). One result of this would be that C transfer would have only a weak relationship to the C demands of the autotrophs, but a stronger one with the assimilation and storage of C by the fungus. This would produce large variations in apparent C transfer among similar plants, with individual plants, by chance, showing exceptionally large C transfers compared with the general trend, exactly as seen by Watkins *et al.* (1996) and Fitter *et al.* (1998).

The story may be intriguingly different for EcM networks. AM and EcM symbioses have in common only that they involve plants and fungi. AM and EcM fungi differ in their evolution, taxonomy, physiology, and ecology. There is, for example, evidence that at least some EcM fungi (typically basidiomycetes) can acquire nitrogen (N) from organic sources and transfer this to plants (Finlay *et al.*, 1992). This is something that the Glomalean fungi which form AM cannot do. It may be that EcM fungi have mechanisms for organic N transfer that can result in large amounts of C moving in combination with the N from fungus to plant. In AM associations, by contrast, C movement is exclusively from plant to fungus. The suggestion that C and nutrient (mainly phosphorus) movement are spatially dislocated in AM associations (Smith and Smith, 1996) adds support to this view.

Although the possibility that plant communities are linked and interact as super-organisms is intriguing (Read, 1997), it must first be established unequivocally that the necessary conditions are met: at present they are not for AM networks, because there is no fungus-to-plant C transfer. The case for EcM networks remains unproven.

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