Carbon transfer between *Quercus leucotrichophora* and *Pinus roxburghii* through ectomycorrhizal mycelial connections

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Both pine and oak received carbon isotopes from their neighbours, indicating that C transfer between pine and oak was bidirectional. Pine received 57% more ^{14}C and 88% more ^{13}C from oak than vice versa. Bidirectional C transfer between oak and pine was 10 times higher in mycorrhizal seedlings than nonmycorrhizal ones. Severing had significant effect on the amount of isotope transferred between oak and pine seedlings and 50% reduction in isotope transfer between oak and pine was observed in severing treatment over that where hyphae were left intact. In nonmycorrhizal treatment, net transfer was more towards oak and in mycorrhizal treatment, both severed and intact, towards pine. Net transfer was about 15 times greater where hyphae were left intact than where they were severed.

ECTOMYCORRHIZAL fungi show a low level of host specificity. A consequence of this is that hyphae growing from root-to-root and plant-to-plant form a mycelial network capable of connecting both intra- and inter-specific combinations of their host plants. The functional significance of such a network, in terms of its capacity to act as a direct pathway for inter-plant carbon, mineral or water transfer, has been considered recently by a number of workers[1–7].

In a study on relationship among seedling growth, soil moisture and cultural practice (pure vs mixed), Bargali and Singh[8] observed that the Central Himalayan subtropical pine (*Pinus roxburghii*) seedlings had more biomass in the presence of banj oak (*Quercus leucotrichophora*) seedlings than in the presence of conspecific seedlings. They speculated that mycorrhizae played a role in nutrient distribution, which could result in better growth of pine seedlings in the presence of oak seedlings. It may be possible that the competitive balance of these two species is affected by below-ground carbon and nutrient transfer, because pine and oak share several ectomycorrhizal species[9,10]. The receiver plant could benefit from this transfer by obtaining mineral nutrients from the fungus whose C was supplied by the donor plant and/or by directly obtaining C or organic nutrients via mycorrhizal links from the donor plant[11]. Other possible consequences of hyphal interconnections include high possibility of seedling radical colonization by fungal mycelium already associated with other plants in a forest[12–14]; transfer of nutrients from dying to living roots thus bypassing the soil pool and alteration of competitive interactions among plants[11,15]. In contrast to ^{14}C, the stable isotope ^{13}C has had limited use as a tracer in ecological research.

Although transfer of nutrients between mycorrhizal plants has been demonstrated, its ecological significance has been questioned. The debate centres on whether the extent of net transfer from one plant to another is sufficiently large to affect significantly plant survival, growth and fitness[11,15], because the transfer is sometimes too small[14,16] or too slow[17] to affect their nutrient status.

The present study addresses these questions regarding the extent of bidirectional and net transfer between oak and pine with shared ectomycorrhizae.

Soil was collected from a site (Khailakhan) originally under banj oak forest, about 2 km from Nainital, around 1900 m altitude. The site is presently occupied by mixed oak and pine forest. The soil type is sandy clay with pH 6.2, water-holding capacity 40%, organic carbon 2.23% and total nitrogen 0.14%. In February 1998, mineral soil was collected to 15-cm depth from five sample points randomly located in the forest. These five samples were combined and sieved through a 5 mm sieve and mixed with white sand (1 : 1) before use.

Mycorrhizal and non-mycorrhizal seedlings of oak and pine were produced from surface-sterilized seeds (4% H_2O_2 for 10 min) in the plastic tray (2 × 1 × 0.5 ft) containing double autoclaved (at 15 lb for 30 min, non-mycorrhizal) and unautoclaved (mycorrhizal) soil : sand mixture (1 : 1 v/v) collected from Khailakhan site.

To establish mycorrhizal hyphal connection, mycorrhizal seedlings of one species were transplanted with non-mycorrhizal seedlings of the other species (mycorrhizal oak–non-mycorrhizal pine and non-mycorrhizal oak–mycorrhizal pine) separated by a nylon filter (pore size 20 μm), which allows only mycorrhizal hyphae to pass through[18]. The pots were grown in the glasshouse for eight months before labelling.

To find the ability of oak and pine mycorrhizal fungi to infect their neighbours and to conform the establishment of hyphal connections between these two species, five pots each of the two different species combinations, viz. mycorrhizal oak–non-mycorrhizal pine and non-mycorrhizal oak–mycorrhizal pine in two-chambered pots, where both chambers were separated by a nylon filter (pore size 20 μm), were established separately and mycorrhizal infection was observed on the roots of non-mycorrhizal member of each pot. Roots of all non-mycorrhizal seedlings were found infected with mycorrhizal fungi after four weeks.

Seedlings were labelled with ^{14}C and ^{13}C using 1 h pulse and 6d (≈ 144 h) chase period. The pulse and chase period occurred under high intensity light inside a high-ventilating fume hood. The steel frame constructed inside the fume hood[5] was large enough to pulse six pots at once (considered as one group): one replicate from each

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treatment. Thirty pots were pulse-labelled each day (five from each treatment) and pulse-labelling was completed in three days. Seedlings of each group were provided with a 144 h chase period.

The study included three mycorrhizal treatments, viz. non-mycorrhizal (control), mycorrhizal but severed, and mycorrhizal and unsevered, with two labelling schemes in a 3×2 factorial treatment in a complete randomized design. There were 15 replicates in each treatment, making a total of 90 pots. The three mycorrhizal treatments were selected to distinguish between inter-specific isotope transfer through all possible pathways: leaching through roots of one species and taken-up by the other, leaching through mycorrhizal and non-mycorrhizal roots and taken-up by mycorrhizal and non-mycorrhizal roots of the other, and mycorrhizal-facilitated inter-specific transfer between two species. In unsevered treatment, the hyphal network connecting roots of oak and pine seedlings were left intact and in the severed one, the hyphae growing between the two compartments were severed with a razor blade 10 min before labelling and the process was repeated once in 24 h throughout the chase period.

The oak and pine seedlings in each pot were pulse-labelled with different isotopes: one with ^{13}C and other with ^{14}C to detect the C isotopes received by one seedling from the other. The seedlings pulse-labelled with a particular isotope were referred to as 'donor' and those which received that same isotope were referred to as 'receiver'. Because of differences in the amount of ^{13}C and ^{14}C that were pulsed, two labelling schemes were applied to each of the three mycorrhizal treatments. For 15 replicates in each treatment, oak (QL) was labelled with ^{14}C and pine (PR) with ^{13}C (labelling scheme called 14QL–13PR). For the other 15 replicates, the reciprocal scheme was applied (13QL–14PR).

Immediately before labelling, each shoot was sealed inside a flexible air-sampling bag (Norton Performance Plastics, Akron, Ohio). Each sampling bag was fitted with a silicone septum in a polypropylene housing for injections with a hypodermic needle. The shoot of one partner seedling was pulse-labelled with 50 ml of $^{13}CO_2$ (gas) (^{13}C 99%, equivalent to 2.25 mmol, 29.25 mg ^{13}C) and at the same time, the shoot of the other partner seedling was pulse-labelled with $^{14}CO_2$ (gas) released from 50 µci $Na_2{}^{14}CO_3$ (1.85 MBq, equivalent to 13.22 µg ^{14}C) with lactic acid. The ratio of pulse mg^{13}C : mg^{14}C was 2548; a greater amount of ^{13}C than ^{14}C was used because of higher detection limits for the stable isotope.

After the 1 h pulse, the labelling bags were removed to release residual $^{13}CO_2$ and $^{14}CO_2$ into the high-venting fume hood and left inside it during the 144 h chase period. During this period, a 16 h/8 h light/dark cycle was maintained with a light intensity of 800 µmol m^{-2} s^{-1} at seedling height. Seedlings were watered daily.

At the end of the chase period, seedlings were harvested and separated into four tissue fractions: leaves, stem, woody root (> 1 mm diameter) and fine root (< 1 mm diameter). At the same time, individual seedlings grown for natural abundance determinations were harvested and separated into four tissue fractions. The tissues were oven-dried at 80°C for 48 h, weighed and finally ground. A sample of 1 mg of each tissue fraction was combusted for %C and analysed for ^{13}C abundance by mass spectrometry using an Automated Nitrogen Carbon Analyzer–Mass Spectrometer (ANCA; Europa Scientific Ltd). The ANCA was modified to exhaust the remaining $^{14}CO_2$ into a vial of NaOH, which was then counted using Liquid Scintillating Counter (Wallace, Finland).

Net photosynthetic rate and specific leaf area were measured on five replicate individuals of oak and pine. Net photosynthetic rate was measured at a PAR of 1000 µmol m^{-2} s^{-1} under ambient CO_2 concentrations on leaf/needle samples at the middle of the shoot. Net photosynthetic rate was measured four times per sample leaf/needle using IRGA (Lycor, 6200). Irradiance (880 ± 76 µmol m^{-2} s^{-1}), air temperature (24 ± 2.5°C), relative humidity (45 ± 7%) and initial CO_2 concentration (400 ppm) were monitored to ensure consistency among samples. Leaf/needle samples were harvested and leaf area (one-sided) measured using leaf-area meter (Lincoln, Nabraska). Biomass was measured after leaves were oven-dried at 80°C for 48 h. Specific leaf area (cm^2 g^{-1}) was calculated as the ratio of leaf area to corresponding leaf weight. Specific leaf area and foliar biomass were used to estimate total leaf area of pot seedlings. Net photosynthetic rates and total leaf area were used to estimate net photosynthetic rates per seedling in the pots.

Samples δ ^{13}C (‰) and ^{14}C (Bq) were converted in mg C isotope for bidirectional and net transfer calculations. The conversions for ^{13}C and ^{14}C were based on procedures described by Boutton[19], and Warembourg and Kummerow[20] respectively. The quantities of ^{13}C present in natural abundance (determined on control plants) were subtracted from ^{13}C of the sample to determine excess mg ^{13}C. Excess mg ^{13}C of the tissue was calculated as the product of excess mg $^{13}C_{sample}$ and $^{14}C_{tissue}$ biomass (mg). Excess mg ^{13}C of the whole plant was determined by adding the excess mg $^{13}C_{tissue}$ of the four tissues. The same procedure was repeated to calculate excess mg $^{14}C_{tissue}$ and of the whole plant. Pulse-labelling efficiency is the ratio of excess mg isotope contained in pulsed seedling tissues at harvest to total mg isotope injected into the chamber.

Bidirectional (sum of isotope received by both species in a pot) and net (differences between isotope received by both species in a pot) C transfer was calculated by the method of Simard *et al.*[5]. For example, for the labelling scheme 14QL–13PR: bidirectional C transfer (BT) = PR excess mg $^{14}C_{plant}$ + QL excess mg $^{13}C_{plant}$, Net C transfer (NT) = PR excess mg $^{14}C_{plant}$ – QL excess mg $^{13}C_{plant}$.

Positive net transfer indicates that a greater amount of isotope was received by pine than by oak and negative transfer indicates the opposite.

Net photosynthetic rates of individual seedlings were compared between species using *t*-test ($n = 5$). For seedlings grown in C transfer pots, data were pooled across all treatments (three mycorrhizal treatments and two labelling schemes) to compare total biomass between species using *t*-test ($n = 90$). Data were then pooled across mycorrhiza treatments alone, and isotope content (^{13}C or ^{14}C) per seedling was compared between species using *t*-test ($n = 45$). Using the pooled data, isotope content (^{14}C or ^{13}C) was also compared among tissues within a species using one-factor ANOVA ($n = 45$). Isotope content (^{14}C or ^{13}C) per seedling was then compared between species mycorrhizal treatments ($n = 15$) using two-factor ANOVA. Bidirectional transfer (^{14}C or ^{13}C) was compared between mycorrhizal treatments using *t*-test ($n = 30$).

To compare relative bidirectional and net transfer between the three mycorrhizal treatments within the same pot (i.e. using both isotopes together), the effect of labelling schemes (14QL–13PR vs 13QL–14PR) and mycorrhizal treatments (non-mycorrhizal vs mycorrhizal but severed vs mycorrhizal and intact) on net transfer was first tested using two-factor ANOVA ($n = 15$). Significant effects of the labelling schemes (14QL–13PR and 13QL–14PR) on whole seedling contents of ^{14}C were removed by applying a correction factor (CF) to excess mg ^{14}C on a treatment–species–tissue basis. The CF was the species-specific ratio of excess mg $^{13}C_{tissue}$ to excess mg $^{14}C_{tissue}$ measured in the reciprocal labelling schemes of the same mycorrhizal treatments. For example, excess mg $^{13}C_{tissue}$ received by pine fine root was measured in the reciprocal labelling scheme 14QL–13PR of the same treatment. The treatment–species-tissue specific CF values were averaged over the replicates per labelling scheme. The corrected excess mg ^{14}C value (excess mg $^{14}C \times CF$) was analogous to excess mg ^{13}C-equivalent value. Data were subjected to *t*-test for pairwise comparison of bidirectional and net transfer between mycorrhizal treatments ($n = 30$).

The leaf, stem and root mass of oak seedlings (8-month-old) was respectively, 1.5 ($P < 0.05$), 2.5 ($P < 0.01$) and 1.6 ($P < 0.05$) times greater than that of pine, but their root : shoot ratios were not significantly different ($P > 0.05$, Table 1). The leaf net photosynthetic rate (PAR = 1000 µmol m^{-2} s^{-1}) of individual oak seedlings was over twice that of pine ($P < 0.01$). Oak leaves had greater specific leaf area than did those of pine ($P < 0.05$). Leaf net photosynthetic rate, specific leaf area and foliage mass were used to calculate leaf area and net photosynthetic rate per seedlings, which were 2.2 and 4.5 times greater respectively, for oak than pine (both $P < 0.01$). Inoculation by mycorrhizal fungi had significant effect on seedlings of both species. Compared to non-inoculated seedlings, the inoculated seedlings had 1.4, 1.3, 1.5 and 1.4 times greater total foliage, stem, root and leaf net photosynthetic rate (all $P < 0.05$) respectively, and 1.5, 2.0 and 2.8 times greater specific leaf area, whole seedling leaf area and whole seedling net photosynthesis, respectively (all $P < 0.01$). Inoculation by mycorrhizal fungi had similar effect on oak and pine seedlings for various parameters, except for net photosynthetic rate of leaf and whole seedling, which increased much more in pine than in oak seedlings. Seedlings with mycorrhizal inoculation had a greater specific leaf area than those without inoculation. This may be related to a greater availability of water and nutrients to inoculated seedlings.

Pulse-labelling resulted in mean ^{14}C and ^{13}C content of 2.77 µg and 3.01 mg per donor seedlings respectively, with no differences between species (Figure 1). Significant difference between mycorrhizal seedling (3.31 µg ^{14}C and 3.51 mg ^{13}C in severed, and 4.00 µg ^{14}C and 4.38 mg ^{13}C in unsevered) was observed over non-mycorrhizal seedlings (1.00 µg ^{14}C and 1.12 mg ^{13}C; Figure 2). This represented average pulse-labelling efficiencies of 7.6, 25 and 30.3% of ^{14}C in non-mycorrhizal, mycorrhizal but severed, and mycorrhizal and intact seedlings respectively, and 3.8, 12 and 14.9% of ^{13}C in non-mycorrhizal, mycorrhizal but severed, and mycorrhizal and intact seedlings respectively.

Pulse-labelled pine roots contained 41.7% of whole seedling ^{14}C content and oak roots contained 48.4% ($P > 0.05$, Figure 3). For seedlings pulse-labelled with ^{14}C, the isotope was more favourably distributed for below-ground export from both pine and oak, when the seedlings were mycorrhizal. Non-mycorrhizal seedlings of oak and pine pulsed with ^{14}C retained most of the isotope in their foli-

Table 1. Net photosynthetic rate, specific leaf area and biomass of 8-month-old pine and oak seedlings

	Non-inoculated		Inoculated	
	Pine	Oak	Pine	Oak
Foliage (g seedling^{-1})	0.20 ± 0.01*	0.28 ± 0.02	0.26 ± 0.01	0.39 ± 0.01
Stem (g seedling^{-1})	0.08 ± 0.01	0.22 ± 0.02	0.12 ± 0.01	0.28 ± 0.01
Root (g seedling^{-1})	0.28 ± 0.01	0.42 ± 0.01	0.38 ± 0.01	0.67 ± 0.03
Total (g seedling^{-1})	0.56 ± 0.02	0.92 ± 0.02	0.76 ± 0.02	1.35 ± 0.02
Root : shoot ratio	1.03 ± 0.05	0.86 ± 0.04	1.02 ± 0.07	1.00 ± 0.05
Leaf net photosynthetic rate (µmol m^{-2} s^{-1} at PAR = 1000 µmol m^{-2} s^{-1})	1.3 ± 0.18	3.8 ± 0.36	2.6 ± 0.21	4.6 ± 0.32
Specific leaf area (cm^{-2} g^{-1})	153.8 ± 7.33	216.2 ± 16.0	212.0 ± 14.8	326.2 ± 10.5
Whole seedling leaf area (cm^{-2} seedling^{-1})	29.8 ± 2.0	61.8 ± 7.9	55.8 ± 6.3	128.7 ± 7.3
Whole seedling net photosynthesis (mmol ws^{-1})	39.7 ± 5.9	228.0 ± 24.8	141.9 ± 12.6	599.7 ± 67.3

*Mean ± 1SE, $n = 5$.

age and stem ($P < 0.01$, Figure 3), but was equally distributed in mycorrhizal root and shoot portions. By contrast, seedlings pulsed with ^{13}C translocated 71.8% to pine roots and 75.2% to oak roots ($P < 0.05$ for both species), of which 60–80% occurred in fine roots at the end of chase period ($P < 0.01$ for both species), with no significant difference between mycorrhizal and non-mycorrhizal seedlings. Consequently, pulse-labelling with ^{13}C resulted in favourable isotope distribution for C transfer from the roots of either species to neighbouring seedlings. The greater proportion of ^{13}C than ^{14}C distributed to roots of donor seedlings resulted from the large $^{13}CO_2$ pulse.

Both pine and oak seedlings received isotopes from each other, indicating that C transfer between pine and oak was bidirectional (Figure 4). The amount of ^{14}C received by oak and pine represented on average 2.2 and 4.8% respectively, and ^{13}C received by oak and pine represented on average 26.5 and 72.0% respectively, of their own isotope fixation (Figure 4). But pine received 57% more ^{14}C ($P < 0.05$) and 88% more ^{13}C ($P < 0.01$) from oak than vice versa (Figure 4). These results indicate that transfer between oak and pine was bidirectional, but pine received significantly more isotope from oak than vice versa.

The distribution of ^{14}C and ^{13}C among tissues of receiver seedlings was similar between species ($P < 0.05$, Figure 4). Transferred ^{14}C did not differ significantly among tissues of either species ($P > 0.05$). A greater proportion (35–70%) of ^{14}C and ^{13}C that reached pine and oak was transferred to shoots in non-mycorrhizal seedlings than mycorrhizal ones (22–38%, $P < 0.05$), with no effect of severing in mycorrhizal seedlings ($P > 0.05$). These results indicate that both ^{14}C and ^{13}C were readily translocated to all tissues of receiver seedlings.

Bidirectional C transfer between oak and pine, whether calculated using ^{14}C alone or ^{14}C and ^{13}C together, was ten times higher in mycorrhizal seedlings than non-mycorrhizal ones ($P < 0.01$, Figure 5). We estimated that bidirectional ^{14}C transfer between the two species (from oak to pine and vice versa) accounted for 3.25% of total ^{14}C assimilated by them (sum of oak and pine ^{14}C assimilation). Severing had significant effect on the amount of isotope transferred between oak and pine seedling, and 50% reduction in isotope transfer between oak and pine (in terms of ^{13}C-equivalent) was observed in the severing treatment over the treatment where hyphae were left intact ($P < 0.05$; Figure 5).

Net transfer was negative in non-mycorrhizal treatment, suggesting a tendency for oak to receive more isotope than pine when seedlings were non-mycorrhizal. However, it was positive in mycorrhizal treatments, both severed and intact. When analysed separately, net transfer (in terms of ^{13}C-equivalent) was approx. 15 times greater where hyphae were left intact than where they were severed ($P < 0.01$; Figure 5).

Transfer of C isotopes between oak and pine was bidirectional, supporting observations by Newman[11] and Simard *et al.*[4,6] that results of one-way labelling studies do not necessarily prove net movement to the receiver plant. Although both ^{14}C and ^{13}C are used to detect bidirectional transfer within pots, ^{14}C alone was used to estimate the magnitude of bidirectional ^{12}C transfer because large $^{13}CO_2$ pulse affects ^{13}C tissue allocation patterns[4–6]. Based

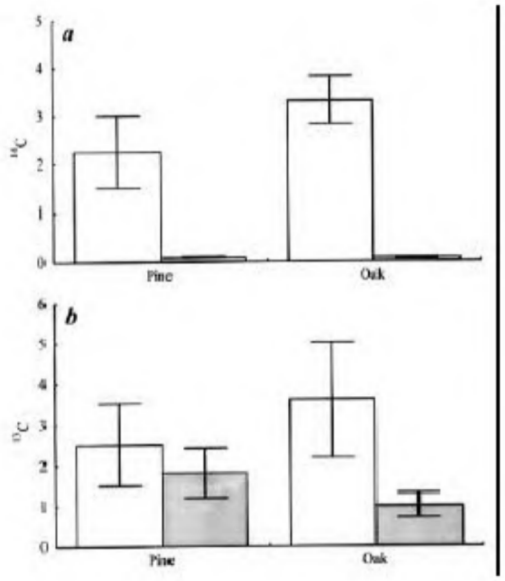

Figure 1. Isotope content of donor (□) and receiver (▨) pine and oak seedlings pulse-labelled with ^{14}C (μg, ***a***) and ^{13}C (mg, ***b***). Error bars are 1 SE; $n = 5$.

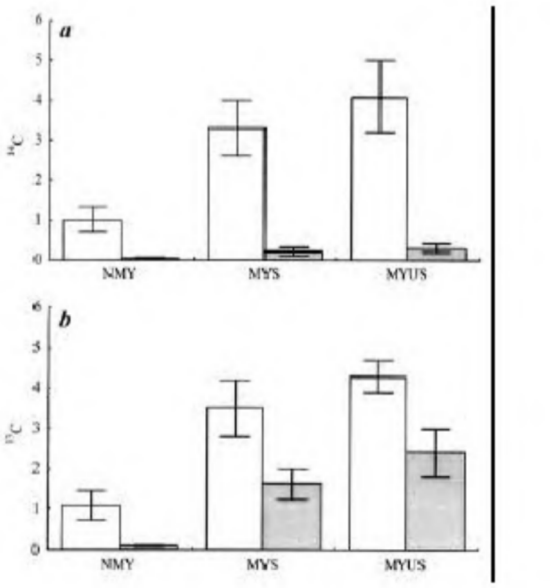

Figure 2. Isotope content of donor (□) and receiver (▨) non-mycorrhizal (NMY) and mycorrhizal seedlings where interconnecting hyphae were intact (MYUS) and severed (MYS) pulse-labelled with ^{14}C (μg, ***a***) and ^{13}C (mg, ***b***). Error bars are 1 SE; $n = 5$.

on ^{14}C alone, bidirectional transfer between oak and pine in our experiment represented on average 3.25% of total ^{14}C assimilated by both species together. Results were comparable to earlier studies by Simard *et al.*[6] and Simard[3], where bidirectional C transfer between paper birch and Douglas-fir seedlings represented 5 (laboratory condition) and 4.7% (field condition) of total isotope assimilated by donors respectively. These comparisons suggest that the laboratory experiments provided a realistic estimate of isotope transfer in plant communities in the field.

In the present study, the amount of ^{14}C and ^{13}C transferred to pine alone represented on average 4.8 and 72% respectively, of that fixed by photosynthesis and the amount transferred to oak alone represented 2.2 and 26% respectively, of its own photosynthesis. Conversely, one-way transfer of ^{14}C and ^{13}C to pine alone presented 3.4 and 49.9% respectively, and to oak alone represented 3.1 and 38.2% respectively, of isotope fixation by donor oak and pine. These results suggest that pine has more to gain than does oak from interspecific C transfer. The results of ^{14}C transfer are similar to those of Simard *et al.*[4] (4–6%), but ^{13}C transfer far exceeds the values reported earlier[4] (4–10%). In this study, hyphal connections might have been formed by more than one fungal species, possibly providing multiple transfer pathways, resulting in ^{14}C one-way transfer close to that reported by Simard *et al.*[4,5], which was 4–6%, in contrast to other reports of 1% or less[1,14,16,21], in which transfer occurred through a single fungal species (unit mycelium). The 'fungal community concept', where several hosts are interconnected by several fungal species, more probably reflects the natural condition of mycorrhizal communities than does the 'unit mycelium concept'[15].

The doubt about C transfer, either directly through ectomycorrhizal fungi or indirectly through soil pathway, encountered by Simard *et al.*[5], was overcome in this experiment by quantifying C transfer in three treatments, viz. reciprocal transfer between non-mycorrhizal individuals, i.e. indirect transfer through root–soil–root pathway; reciprocal transfer between mycorrhizal but severed individuals, i.e. indirect transfer through mycorrhizal root–soil–mycorrhizal root pathways, and reciprocal transfer between two individuals interconnected by mycorrhizal mycelium, i.e. direct hyphal transfer through root–mycorrhizal mycelia–root pathway. The presence of hyphal connections between the two species is evidenced by two observations. First, by planting non-mycorrhizal individuals of one species with the mycorrhizal individuals of the other species in a pot separated by a membrane before the main experiment, separately. The presence of mycorrhizal fungi on the roots of the non-mycorrhizal partner provides direct evidence of hyphal connections between two individuals. The method by Francis and Read[14] to confirm hyphal connections by autoradiography was not

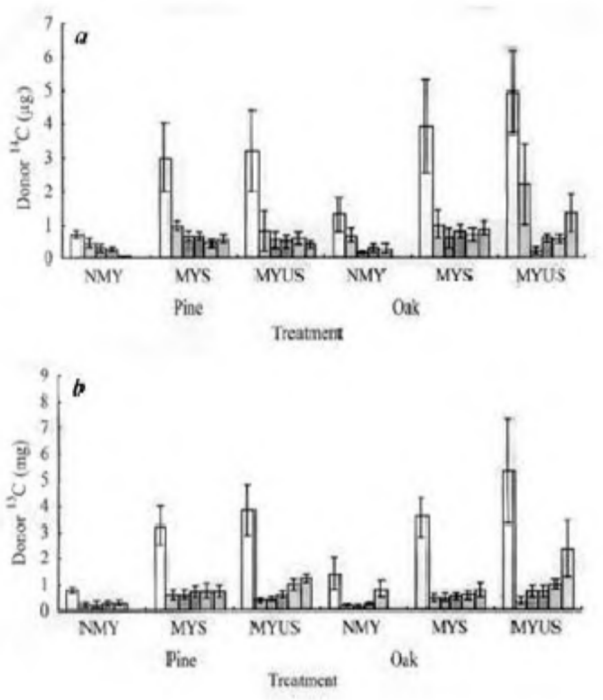

Figure 3. Total and tissue (total; foliage; stem; coarse root; non-mycorrhizal fine root and mycorrhizal fine root) isotope content of donor pine and oak seedlings pulse-labelled with ^{14}C (***a***) and ^{13}C (***b***). Error bars are 1 SE; $n = 5$.

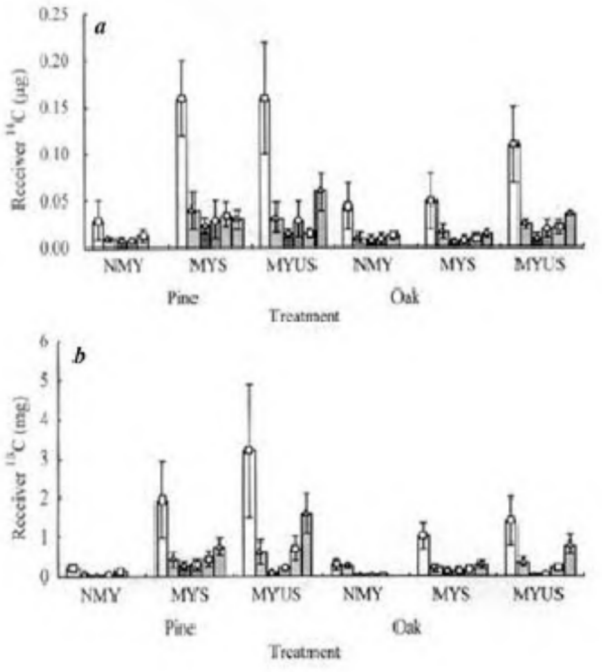

Figure 4. Total and tissue (total; foliage; stem; coarse root; non-mycorrhizal fine root and mycorrhizal fine root) isotope content of receiver pine and oak seedlings pulse-labelled with ^{14}C (***a***) and ^{13}C (***b***). Error bars are 1 SE; $n = 5$.

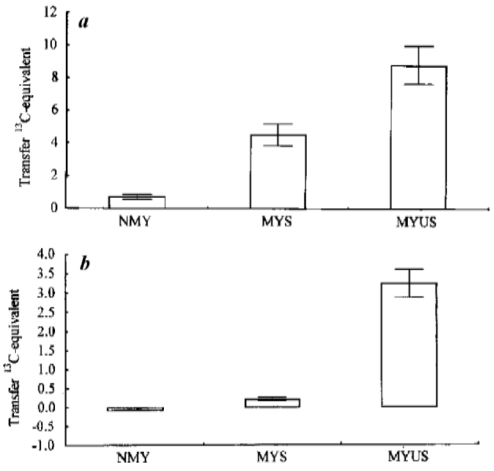

Figure 5. Bidirectional (***a***) and net (***b***) transfer between non-mycorrhizal and mycorrhizal pine and oak seedlings where interconnecting hyphae were intact and severed. Total amount of isotope transferred is expressed as my ^{13}C-equivalent. Error bars are 1 SE; $n = 5$.

used in this study. Secondly, differences in C transfer were observed between intact (i.e. through ectomycorrhizal connections plus soil pathways) and severed pots (i.e. through soil pathways alone). In the experiment done by Simard *et al.*[5], in spite of 40% greater bidirectional and three times higher net transfer where hyphae were left intact than when they were severed immediately before labelling, the difference was not significant because of low replicate number ($n = 3$) in each treatment and high variability among replicates. To find a significant difference in these two treatments, more replicates ($n = 15$) in each treatment were maintained in the present study. Additionally, pots in mycorrhizal and severed treatments were regularly (once in 24 h) severed throughout the chase period to reduce the experimental error due to anastomosis and partial connections of mycorrhizal hyphae in these pots, which was not done by Simard *et al.*[4,5].

As with directional transfer, these results suggest that interconnecting hyphae might have played an important role in facilitating C transfer between pine and oak. Pine received 57% more ^{14}C and 88% more ^{13}C from oak than oak received from pine. Net transfer estimates based on ^{13}C-equivalent (^{13}C and corrected ^{14}C values) were positive, suggesting a tendency of pine to receive more isotope from oak than vice versa, which was over 15 times greater where hyphae were left intact than when they were severed before labelling.

The direction and extent of inter-plant transfer is thought to be influenced by source–sink relationships between plants, such as those established by differences in net photosynthesis rate, nutrient status, etc.[22–24]. In general, leaves of broadleaved tree species have faster photosynthetic rate than conifers[25], as a result of differences in their diffusion pathway for CO_2. The combined differences in net photosynthetic rate, tissue nutrient concentration and C allocation patterns between oak and pine may have contributed to the tendency of pine to receive more isotope than oak in this study and provide a source–sink mechanism for net transfer from oak to pine. However, net transfer between any two species is a complex process which needs further investigation. It could either be governed by the relative sink strength of the host seedling alone (an active process) or, due to differences in net photosynthetic rate and nutrient status (i.e. along the concentration gradient, a passive process) or a combination of both.

Whether the observed bidirectional and net C transfer between pine and oak is significant to seedling performance or fitness, or how the seedlings interact over a long time period, cannot be determined from our study. Carbon transfer of this magnitude, whether momentary or prolonged, could affect seedling performance under particularly stressful conditions or over the lifetime of a seedling, especially in a mixed population.

1. Finlay, R. D. and Read, D. J., The structure and function of the vegetative mycelium of ectomycorrhizal plants. I. Translocation of ^{14}C-labelled carbon between plants interconnected by a common mycelium. *New Phytol.*, 1986, **103**, 143–156.
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Relationship between environmental factors and diurnal variation of bioelectric potentials of an intact jute plant

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The effect of environmental factors like photosynthetic photon flux density, air and soil temperature on the inherent bioelectric potential of intact jute plants measured at two points 100 cm apart was found to vary between – 18 mV at night and 40 mV during midday. The bioelectric potential of the plant was more closely related to soil temperature than air temperature or light intensity.

ENVIRONMENT–plant interaction is fundamental to growth and development of an organism. The major environmental factors – light intensity, air, soil temperature and humidity, have profound influence on physiological processes. Physiological mechanisms for rapid long-distance communication between plant tissues and organs, particularly in response to such external stimuli, are poorly understood[1]. The translocation of phytohormones or other endogenous organic and inorganic compounds has been traditionally viewed as the primary means of signalling between stimulated and specific remote tissues in the plant where physiological responses are observed, but it has relatively slow response kinetics. Many systemic physiological responses within higher plants are known to occur within seconds of treatment with specific stimuli[2], much quicker than diffusion phenomena. The process of electrical changes associated with rapid propagation is not clear, although much is known about the bioelectric potential of plant cells and their possible sources in a living system[3,4]. The primary source for the transmembrane potential difference is the electrochemical process of excitable cells[5,6]. In multicellular systems, the conductive tissues of vascular plants not only facilitate the movement of electrolytes and other physiological substances, but also actuate the transfer of bioelectric impulses[7]. Thus, the measurement of electrical potential difference of an intact plant may largely reflect the picture of electrophysiological status of the plant at a given time and condition. Moreover, as electrical potential differences are expressed spatially within biological tissue and are modulated over time, some investigators have postulated the involvement of electrical potential in inter- and intracellular communication[8–13]. The use of electrophysiological investigation with microelectrode technique, which can determine the kind of cells that generate action potential

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