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Reciprocal transfer of carbon isotopes between ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*

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SUMMARY

Interspecific C transfer was studied in laboratory microcosms containing pairs of 6-month-old *Betula papyrifera* Marsh. and *Pseudotsuga menziesii* (Mirb.) Franco seedlings growing in individual, root-restrictive (28 μm pore size) pouches filled with field soil. Interspecific transfer was examined by reciprocal labelling of seedlings with $^{13}CO_{2(gas)}$ and $^{14}CO_{2(gas)}$. At the time of labelling, the root zones of ectomycorrhizal (EM) *B. papyrifera* and *P. menziesii* were interconnected by an extensive network of EM mycelium. Carbon transferred through EM connections was distinguished from that through soil pathways by comparing microcosms where interconnecting hyphae were left intact vs. those where they were severed immediately before labelling.

Transfer was bidirectional, and represented 5% of total isotope uptake by both *B. papyrifera* and *P. menziesii* together. *P. menziesii* received on average 50% more ^{14}C and 66% more ^{13}C from paper birch than vice versa, however, differences between species were not statistically significant. Neither net nor bidirectional transfer differed between severing treatments, leaving in question the relative importance of EM hyphae versus soil transfer pathways. The tendency for *P. menziesii* to receive more isotope than *B. papyrifera* corresponded with a 10-fold greater net photosynthetic rate per seedling and two-fold greater foliar N concentration of *B. papyrifera* than *P. menziesii*.

Key words: Ectomycorrhiza, carbon transfer, carbon isotope, *Pseudotsuga menziesii* (Douglas fir), *Betula papyrifera* (paper birch).

INTRODUCTION

The low host specificity for arbuscular mycorrhizal (AM) fungi and many ectomycorrhizal (EM) fungi can result in hyphal connection between host plants (Molina, Massicotte & Trappe, 1992); this has been demonstrated using ^{14}C-labelling, macro-autoradiography (Brownlee *et al.*, 1983; Francis & Read, 1984; Finlay & Read, 1986) and direct observation (Newman *et al.*, 1994). One of several possible consequences of hyphal interconnections is direct transport of C, minerals, or water between plants. Interconnecting mycorrhizal fungi have been demonstrated to facilitate interplant transfer of the isotope tracers ^3H_2O (e.g. Duddridge, Malibari &

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Read, 1980), ^{14}C (e.g. Francis & Read, 1984),^{13}C (Watkins *et al*., 1996), ^{32}P (e.g. Newman & Eason, 1993), and ^{15}N (e.g. Arnebrant *et al*., 1993). 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 (Newman, 1988). Other possible consequences of hyphal interconnections include: greater or more rapid infection of seedlings that grow into contact with established plants; transfer of nutrients from dying to living roots thus by-passing the soil pool; and alteration of competitive interactions between plants (Newman, 1988; Miller & Allen 1992).

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 or fitness (Newman, 1988; Miller & Allen, 1992). Several problems are as follows. Isotope transfer has been very small (^{14}C) or very slow (^{32}P) in most studies. For example, (i) only 0·1 % of donor ^{14}C was transferred from *Plantago lanceolata* to shaded *Festuca ovina* via AM hyphae (Francis & Read 1984), (ii) 0·2–1·0 % of donor ^{14}C was transferred between *Pinus contorta* seedlings, with more in shade than sun (Read, Francis & Finlay, 1985), (iii) rate of ^{32}P transfer between mycorrhizal-connected *Lolium perenne* was too slow to affect their nutrient status (Newman & Eason, 1993), and (iv) mycorrhiza influenced N status of corn (*Zea mays*) more through improved P uptake than through direct ^{15}N transfer from the neighbouring legume, *Glycine max* (Hamel & Smith, 1992). In contrast to these examples, however, Arnebrant *et al*. (1993) found that 5–15 % of $^{15}N_{2(gas)}$ fixed by the *Alnus glutinosa–Frankia* sp. association was transferred to *Pinus contorta* through EM (*Paxillus involutus*) connections, and that *c*. 20 % of the nitrogen found in pine was derived from N_2-fixation.

Another problem is that net transfer from one plant connected to another has yet to be proven – that is, whether gain in material by one plant exceeds that of its connected neighbour. Usually one plant has been fed an isotope and its neighbour assessed a few days later, thereby quantifying one-way transfer. Although Arnebrant *et al*. (1993) quantified only one-way transfer of ^{15}N from N_2-fixer *A. glutinosa* to *P. contorta*, they suggest that the large amounts transferred and the fact that *P. contorta* is non-N_2-fixing imply net transfer. In a novel experiment using natural abundance of ^{13}C as a 'natural tracer', Watkins *et al*. (1996) examined bidirectional transfer between AM *Plantago lanceolata* (C_3 plant) and *Cynodon dactylon* (C_4 plant) over a 10-wk period, but could not determine whether net transfer occurred. For ^{14}C-labelled photosynthate, Jakobsen (1991) suggests that net or bi-directional transfer can only be determined by comparing the results of reciprocal labelling in two parallel experiments. If net transfer does occur, its significance could be evaluated by comparing the net amount transferred with the receiver plants' gain by photosynthesis (Newman, 1988).

Net transfer can be expected to occur if connected plants differ in some way (such as net photosynthetic rate, nutrient status, or N_2-fixing capability) that establishes a source–sink relationship (Read *et al*., 1985; Newman, 1988; Bethlenfalvay *et al*., 1991; Arnebrant *et al*., 1993). For example, (i) Francis & Read (1984) established a photosynthate concentration gradient by shading *F. ovina* seedlings that were connected to mature *P. lanceolata* via AM hyphae, and showed that over six times more ^{14}C was transferred to shaded than unshaded seedlings, (ii) ^{15}N transfer via EM or AM hyphae has been shown to occur from nodulated N_2-fixing to non-N_2-fixing plants, which differ in tissue N contents (Bethlenfalvay *et al*., 1991; Frey & Schuepp, 1992; Hamel & Smith, 1992; Arnebrant *et al*., 1993; Ekblad & Huss-Danell, 1995), and (iii) fertilization of donor plants increased mycorrhizal-mediated ^{32}P transfer to unfertilized receiver plants (Ritz & Newman, 1986; Eissenstat, 1990).

Interplant transfer also occurs, however, where there is no apparent source–sink relationship between donor and receiver plants. Transfer of ^{14}C and ^{32}P, for example, has been shown to occur between connected plants of the same species, size, age, and nutrient status (Read *et al*., 1985; Ritz & Newman 1986). Waters & Borowicz (1994) tested the effect of source–sink relationships on magnitude and direction of net C transfer between pairs of *Lotus corniculatus* linked by AM fungi by clipping one plant. They labelled either clipped (sink) or unclipped plants (source) with ^{14}C and showed that net ^{14}C flow was in the opposite direction to that expected; that is, away from clipped plants and toward unclipped plants. They suggested that clipping might increase the concentration of labile C in roots, thereby increasing the diffusion gradient for C out of roots and into mycorrhizal fungi, and then into the mycorrhiza of neighbouring plants. Because neither the concentration nor the compounds of labile C in roots and mycorrhizal fungi were measured, however, there is insufficient evidence that diffusion gradients drive interplant transfer rather than a more complex mechanism that also involves membrane channels or carriers (e.g. Smith & Smith, 1990). Miller & Allen (1992) suggest that extent of hyphal linkages also could influence extent of transfer, even where leaf sinks do not operate. In a tallgrass prairie community, Walter *et al*. (1996) showed that received ^{32}P decreased with increasing distance from donor plants, and they suggest that

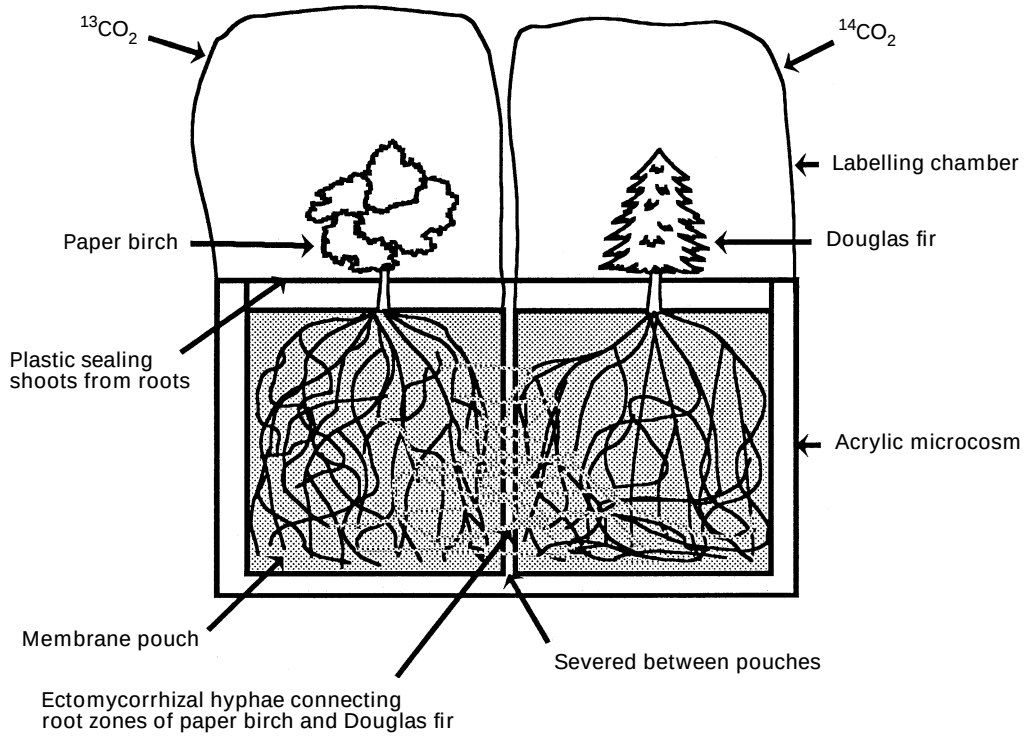

Figure 1. Microcosm and labelling chambers. Two labelling schemes (^{14}C-*Betula papyrifera*↔^{13}C-*Pseudotsuga. menziesii*; ^{13}C-*B. papyrifera*↔^{14}C-*P. menziesii*) and two severing treatments were applied. This figure shows one labelling scheme (^{13}C-*B. papyrifera*↔^{14}C-*P. menziesii*).

frequency of mycorrhizal connections and/or other rhizosphere interactions were important factors controlling the magnitude of interplant transfer. Mycorrhizal fungi themselves may also influence direction of net transfer. For example, mycorrhizal fungi might exchange different sources of C or provide nutrients at different rates to each of the interconnected plants (Miller & Allen, 1992).

As an extension of previous studies, our study addresses questions regarding the extent of bidirectional and net transfer between plants with shared EM fungi. We studied C transfer in microcosms containing EM paper-birch and Douglas-fir seedlings growing in individual, root-restrictive pouches filled with field soil. The two species had previously been shown to share seven ectomycorrhizal morphotypes on 90% of their root tips in a bioassay using the same plant and soil material as we used in this study (Simard *et al.*, 1997*a*). Carbon transfer was studied following reciprocal exposure of one plant to the radioactive isotope ^{14}C and the other plant to the stable isotope ^{13}C.

The objective of our study was to quantify bidirectional and net transfer of C between paper-birch and Douglas-fir seedlings. We hypothesized that isotope transfer is bidirectional, but that more is transferred from paper birch to Douglas fir than vice versa (i.e. net transfer to Douglas fir) because of an interspecific concentration gradient in labile C created by differences in net photosynthetic rate, C allocation patterns and foliar N (Simard, Jones & Durall, 1997*b*).

MATERIALS AND METHODS

Soil collection

Soil was collected from a clearcut site in the North Adams River valley in south-central British Columbia. Before clearcutting in 1987, the site had been occupied by a mature, mixed forest of Douglas fir (*Pseudotsuga menzeisii* (Mirb.) Franco) and paper birch (*Betula papyrifera* Marsh.) The soil type was a Humo-Ferric Podzol (Canadian Soil Survey Committee, 1978), formed over a granitic alluvial blanket. The soil surface layers (to 50 cm) were sandy loam to loamy sand with a coarse fragment content of $< 10\%$.

In September, 1993, mineral soil was collected to 15-cm depth from five sample points randomly located between pairs of out-planted Douglas fir and naturally regenerated paper-birch seedlings. The five samples were combined to make one sample. The composite sample was placed in plastic bags, set on ice in a cooler, and then transported to the laboratory, where it was immediately sieved to 4 mm, homogenized, and mixed (3:1 by volume) with perlite.

Preparation and planting of microcosms

A modification of the 'root-mycocosm' design of Rygiewicz, Miller & Durall (1988) was used to construct 12 acrylic microcosms, each 22 cm high by 20 cm wide and 3 cm thick. Two Nitex® membrane

(Tetko Inc., New York) pouches 20 cm high, 10 cm wide and 3 cm thick, were fitted snugly side-by-side in the root chamber of each microcosm (Fig. 1). The membrane pore size was 28 μm, which has been shown to restrict root but not hyphal penetration (Neufeld *et al*., 1989). Each pouch was filled with the 3:1 soil:perlite rooting medium. Douglas-fir seeds were planted in one and paper birch in the other pouch of each microcosm. Before being planted, Douglas-fir and paper-birch seeds were surface sterilized and stratified in H_2O_2. Five seeds of one species were planted per pouch. Sterile silica sand was spread over Douglas-fir seeds to 0·5 cm depth, and over paper-birch seeds to a bare covering, to stabilize the surface and minimize mortality due to damping-off fungi. The microcosms were wrapped in aluminum foil to ensure darkness and reduce evaporation.

Seedlings were grown in the glasshouse between October, 1993, and March, 1994, under 280 μmol m^{-2} s^{-1} PAR supplied by lights set on a 16 h/8 h light/dark cycle and supplemented by natural light. Temperatures ranged from 24 °C (light) to 18 °C (dark). Microcosms were watered daily and relocated monthly to reduce environmental differences. After 4 wk, seedlings were thinned to one per pouch. After 5 months, each pouch received 50 ml of Peters'® solution (20:20:20 N-P-K). The C transfer experiment was conducted when seedlings were 5 months old.

Individual seedlings of paper birch and Douglas fir were also grown for measurements of foliar nitrogen, specific leaf area, net photosynthetic rate, and ^{13}C and ^{14}C natural abundance. The seedlings were grown in Leach tubes (Ray Leach 'Cone-tainer' Single Cell System, supplied by Stuewe and Sons, Corvallis, Oregon) using the same rooting medium, planting procedures, and glasshouse conditions described above.

Study design

The study included two 'severing' treatments and two labelling schemes in a 2×2 factorial treatment structure with threefold replication applied in a completely randomized design ($n = 12$). The two severing treatments were imposed to distinguish between interspecific isotope transfer through EM fungi and transfer through other pathways. Other possible pathways included respired $CO_{2(gas)}$ as well as root/fungal exudates and sloughed root/fungal cells in irrigation water that was allowed to pass between pouches. In one treatment (unsevered), the hyphal networks connecting the root zones of paper-birch and Douglas-fir seedlings were left intact, and in the other treatment (severed) the hyphae growing between the two pouches were severed with a razor blade 10 min, before labelling. At the time of treatment, Douglas-fir and paper-birch seedlings were ectomycorrhizal and their root zones were interconnected by an extensive network of EM mycelium. Although EM hyphae could be seen passing through the membrane pouches, it is possible that other non-mycorrhizal hyphae were present as well.

The paper-birch and Douglas-fir seedlings in each microcosm were pulse-labelled with different isotopes: one with ^{13}C and the other with ^{14}C. This approach enabled detection of the C isotope that was received by one seedling from the other. The seedlings pulse-labelled with a particular isotope were referred to as 'donors', and those which received that same isotope were referred to as 'receivers'. Because of differences in the amount of ^{13}C and ^{14}C that were pulsed, two labelling schemes were applied to each severing treatment. For three replicates per severing treatment, paper-birch (PB) was labelled with ^{14}C, and Douglas fir (DF) with ^{13}C (labelling scheme called 14PB-13DF) . For the other three replicates, the reciprocal scheme was applied, so paper birch was labelled with ^{13}C and Douglas fir with ^{14}C (13PB-14DF).

^{13}C and ^{14}C labelling procedures

Seedlings were labelled with ^{13}C and ^{14}C using 1-h pulse and 6-d chase periods. A preliminary experiment had determined that the 6-d chase was appropriate for maximum isotope translocation to fine roots (Simard *et al*., 1997*b*). The pulse and chase periods occurred under high intensity light inside a high-venting fume-hood. A 1·2 m × 1·2 m steel frame light stand was constructed inside the fumehood to support a 90 cm × 30 cm × 8 cm open-topped, circulating acrylic water bath at *c*. 1·0 m height. Six ESD projection quartzline multi-mirror bulbs, socket no. 2CX-30 (General Electric, Kennedy Webster, Chicago, IL) were wired 5 cm above the bath, which kept seedling temperatures near 25 °C, and light diffusion plates (variegated plastic) were taped to the bottom of the bath to diffuse the direct beam. The light intensity under the water bath was *c*. 1000 μmol m^{-2} s^{-1}. The steel frame was large enough to pulse four microcosms at once: one replicate of each treatment.

Immediately before labelling, the rooting medium was sealed from shoots using plastic Saran Wrap® and duct tape. Each shoot was then sealed inside flexible, air-tight, 15 cm wide × 15 cm tall, Teflon® Chemware® fluoropolymer (5 mm thick) gas sampling bags (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 for 1 h 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 for 1 h

with $^{14}CO_{2(gas)}$ released from 50 μCi (1·85 MBq, equivalent to 13·22 μg ^{14}C) $Na_{2}{}_{14}CO_3$ with lactic acid. The ratio of pulsed mg ^{13}C : mg ^{14}C was 2213; a greater amount of ^{13}C than ^{14}C was used because of higher detection limits for the stable isotope. We estimate that the $^{13}CO_2$ concentration was 1·4 % by volume inside the labelling chamber, whereas the $^{14}CO_2$ concentration in the other chamber was near ambient levels (*c*. 0·03 % according to Perry, 1994). The large difference in CO_2 concentration between the ^{13}C and ^{14}C pulses might affect photosynthesis and C allocation to plant tissues and mycorrhizal fungi. Consequently the ^{13}C and ^{14}C labellings can be used only to detect reciprocal transfer and the relative effects of severing treatments, rather than to compare isotope allocation patterns directly or to determine absolute amounts of C transferred (see 'Statistical Analysis'). All microcosms were labelled within 4 h of each other (1200 hours–1600 hours), 6 h before the end of the photoperiod (2200 hours).

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. The microcosms remained inside the fumehood during the 6-d chase period. The rapid suction of residual CO_2 from the pulse-labelled donor seedlings minimized unwanted uptake of C isotopes by foliage of receiver seedlings during chamber removal and the chase period. During the chase period in this study, a 16 h/8 h light/dark cycle was maintained, with a light intensity of *c*. 300–400 μmol m^{-2} s^{-1}. Seedlings were watered daily with an eye-dropper inserted into a small port in the seal separating shoots from the rooting medium.

At the end of the chase period, seedlings were harvested and separated into four tissue fractions: leaves, stems, coarse roots (> 1 mm diameter), and fine roots (< 1 mm diameter). At the same time, individual seedlings grown for natural abundance determinations were harvested and separated into the four tissue fractions. The tissues were ovendried at 80 °C for 48 h, weighed, and ground to 20 mesh in a Wiley mill. One sample (1 mg) of each tissue fraction was combusted for %C and analysed for ^{13}C abundance by mass spectrometry using a Europa Scientific ANCA. The Europa Scientific ANCA was modified to exhaust the remaining $^{14}CO_2$ into a vial of NaOH. The NaOH was then counted for ^{14}C using liquid scintillation.

Net photosynthetic rate

Net photosynthetic rate and specific leaf area were measured on three replicate individuals of paper birch and Douglas fir, before the C transfer experiments. The measured seedlings were comparable in size and vigour to those grown in the C transfer microcosms. Net photosynthetic rate was measured at a PAR of 1000 μmol m^{-2} s^{-1} (over the waveband 400–700 nm) and under ambient CO_2 concentrations. As a result, the measurements are an adequate approximation of net photosynthetic rates in the $^{14}CO_2$ labelling chambers, but probably underestimate those in the $^{13}CO_2$-labelling chambers. For each paper-birch seedling, a single attached, fully developed leaf located approx. 1/3 from the top of the crown was randomly sampled. For each Douglas fir, a lateral branch was randomly sampled from the top whorl. Net photosynthetic rate was measured four times per sample leaf or branch using a Li-Cor 6200 infra-red gas analyser (Lincoln, Nebraska). Irradiance (1000 ± 50 μmol m^{-2} s^{-1}), air temperature (21 ± 1 °C), r.h. (16 ± 1 %), and initial CO_2 concentration (385 ppm) were monitored to ensure consistency among samples. Leaves were immediately harvested and leaf area (one sided) measured using a Li-Cor 3100 leaf area meter (Lincoln, Nebraska). 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 microcosm seedlings. Net photosynthetic rate and total leaf area were used to estimate net photosynthetic rates per seedling in the microcosms.

Foliar N

Foliage was collected from the top 1/3 of paper-birch crowns and top whorl of Douglas-fir individuals. Oven-dried foliage was ground to 40 mesh in a Wiley mill. Nitrogen content was determined by combustion using a Leco CHN-600 (Kalra & Maynard, 1991).

Bidirectional and net C transfer

Sample $\delta^{13}C$ (‰) and ^{14}C (Bq) were converted to mg C isotope for bidirectional and net-transfer calculations. The conversions for ^{13}C and ^{14}C were based on procedures described by Boutton (1991) and Warembourg & Kummerow (1991), respectively. The tissue $\delta^{13}C$ values first were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of the sample (R):

$$R_{sample} = {}^{13}C/{}^{12}C = [(\delta^{13}C/1000) + 1] \times R_{standard},$$

where $R_{standard} = 0{\cdot}0112372$ (PDB standard), and $\delta^{13}C$ is parts per thousand (‰) ^{13}C abundance of the sample as determined by mass spectrometry. The fractional abundance (A) of ^{13}C relative to $^{13}C + {}^{12}C$ was then related to R_{sample} by the equation:

$$A = {}^{13}C/({}^{13}C + {}^{12}C) = R_{sample}/(R_{sample} + 1).$$

The product of fractional abundance and total C content (mg) of the sample provided an estimate of the quantity (mg) of ^{13}C in the sample. The quantity of ^{13}C present at natural abundance (determined on control plants) was subtracted from ^{13}C of the sample to determine excess $mg^{13}C_{sample}$. Excess

$mg^{13}C$ of the tissue was calculated as the product of excess $mg^{13}C_{sample}$ and tissue biomass (mg). Excess $mg^{13}C$ of the whole plant was determined by summing the excess $mg^{13}C_{tissue}$ of the four tissue types.

Conversion of $Bq^{14}C$ to $mg^{14}C$ was based on the batch specific activity (λ) of $Na_{2}{}_{14}CO_3$, $\lambda = 1{\cdot}96$ GBq $mmol^{-1}$ (Amersham Canada). First, radioactivity of a sample (Bq or dps) was expressed per mg C ($Bq^{14}C_{mgC}$):

$$Bq^{14}C_{mgC} = Bq^{14}C_{sample}/mgC_{sample}.$$

Radioactive units (Bq) were converted to $mol^{14}C$ using λ, and then mg^{14} C using the atomic weight of ^{14}C ($aw^{14}C$):

$$mol^{14}C_{mgC} = Bq^{14}C_{mgC}/\lambda,$$
$$mg^{14}C_{mgC} = mol^{14}C_{mgC} \times aw^{14}C.$$

As with ^{13}C, excess $mg^{14}C_{mgC}$ of a sample was calculated by subtracting natural abundance values (determined on control plants) from sample values. Excess $mg^{14}C$ of the tissue was calculated as the product of excess $mg^{14}C_{mgC}$ and tissue C, and excess $mg^{14}C$ of the whole plant was determined by summing the excess $mg^{14}C_{tissue}$ of the four tissue types. Pulse-labelling efficiency was the ratio of excess mg isotope contained in pulsed seedling tissues at harvest to total mg isotope injected into the chamber.

Bidirectional and net C transfer were examined using two approaches: (1) analysis of ^{14}C transferred to paper birch and Douglas fir grown in different microcosms, and (2) analysis of both ^{14}C and ^{13}C transferred between paired paper birch and Douglas fir grown in the same microcosm. The first approach, using ^{14}C alone, was used to provide an estimate of bidirectional transfer as a percentage of total isotope assimilated by donor seedlings. Compared with ^{13}C, we consider ^{14}C the more representative tracer of ^{12}C behaviour because the CO_2 concentration of the labelling chamber was not substantially changed by the $^{14}CO_2$ pulse. By contrast, the large perturbation to the microcosms caused by the $^{13}CO_2$ pulse appeared to have altered C allocation and transfer patterns. Using ^{14}C alone, bidirectional transfer (BT) was the sum of ^{14}C received by both species grown in different microcosms (Douglas fir in the 14PB-13DF labelling scheme, and paper birch in the 13PB-14DF labelling scheme):

$$BT = DF \text{ excess } mg^{14}C + PB \text{ excess } mg^{14}C$$

Bidirectional ^{14}C transfer was expressed as a proportion of total ^{14}C assimilated by (*a*) Douglas fir, (*b*) paper birch, and (*c*) Douglas fir and paper birch together.

Whether bidirectional and net transfer occurred between pairs of paper birch and Douglas fir growing in the same microcosm is of central importance to our study objectives. Although the large $^{13}CO_2$ pulse negates our ability to accurately trace *absolute* quantity of ^{12}C transferred within a microcosm, we can compare *relative* transfer between the two severing treatments using both isotopes together. Consequently, we estimated bidirectional and net transfer from the total amounts of excess isotope that were received from partner donor plants growing in the same microcosms. For the labelling scheme 14PB-13DF, paper birch received ^{13}C from Douglas fir at the same time as Douglas fir received ^{14}C from paper birch. Similarly, for the reciprocal labelling scheme 13PB-14DF, paper birch received ^{14}C from Douglas fir while Douglas fir received ^{13}C from paper birch. Bidirectional transfer was the sum of isotope received by both species in a microcosm. For example, bidirectional transfer in a microcosm subject to the labelling scheme 14PB-13DF was calculated as:

$$BT = DF \text{ excess } mg^{14}C_{plant} + PB \text{ excess } mg^{13}C_{plant}.$$

Net transfer was calculated as the difference between isotope received by Douglas fir and that received by paper birch in a microcosm. For example, net transfer (NT) in a microcosm subject to the labelling scheme 14PB-13DF was calculated as:

$$NT = DF \text{ excess } mg^{14}C_{plant} - PB \text{ excess } mg^{13}C_{plant}.$$

Positive net transfer indicates that a greater amount of isotope was received by Douglas fir than by paper birch, and negative net transfer indicates the opposite.

Statistical analysis

Net photosynthetic rate and foliar N concentration of individual seedlings were compared between species using *t*-tests ($n = 3$). For seedlings grown in C transfer microcosms, data were pooled across all treatments (i.e. severing treatments and labelling schemes) to compare total biomass between species using *t*-tests ($n = 12$). Data were then pooled across severing treatments alone, and isotope content (^{14}C or ^{13}C) per seedling compared between species using *t*-tests ($n = 6$). Using the pooled data, isotope content (^{14}C or ^{13}C) was also compared among tissues within a species using one-factor ANOVA ($n = 6$). Isotope content (^{14}C or ^{13}C) per seedling was then compared between species and severing treatments using two-factor ANOVA ($n = 3$). Bidirectional transfer (^{14}C or ^{13}C) was compared between severing treatments using *t*-tests ($n = 6$).

To compare *relative* bidirectional and net transfer between the two severing treatments within the same microcosm (i.e. using both isotopes together), the effects of labelling scheme (14PB-13DF vs. 13PB-14DF) and severing treatment (unsevered versus severed) on net transfer were first tested using two-

Table 1. *Net photosynthetic rate, specific leaf area, biomass and foliar nitrogen of 6-month-old paper-birch and Douglas-fir seedlings*

Seedling characteristic	Paper birch	Douglas fir
Biomass (g d.wt)		
Total‡	1·70 ± 0·11*†	0·83 ± 0·08
Foliage	0·44 ± 0·03	0·22 ± 0·01
Stems	0·11 ± 0·01	0·09 ± 0·01
Coarse roots	0·24 ± 0·02	0·09 ± 0·01
Fine roots	0·91 ± 0·05	0·43 ± 0·03
Root : shoot ratio‡	2·18 ± 0·19	1·77 ± 0·13
Leaf net photosynthetic rate (μmol m^{-2} s^{-1}) at PAR = 1000 μmol m^{-2} s^{-1}§	6·48 ± 0·68	2·92 ± 0·32
Specific leaf area (cm^2 g^{-1})§	131·3 ± 6·4	51·1 ± 0·2
Whole seedling leaf area (cm^2)‡	57·8 ± 4·7	11·2 ± 0·8
Whole seedling net photosynthetic rate (mmol ws^{-1})‡	374·5 ± 30·0	32·1 ± 2·4
Foliar nitrogen concentration (% d.wt)§	2·31 ± 0·03	1·38 ± 0·02

* Mean ± 1SE.

† *t*-test detected that paper birch was significantly greater than Douglas fir for all seedling characteristics tested at $P < 0·01$, except for root : shoot ratio where $P < 0·10$ (d.f. = 11, except for specific leaf area where d.f. = 2).

‡ Seedlings grown in microcosms.

§ Individual seedlings grown in Leach tubes.

factor ANOVA ($n = 3$). Whole seedling contents of ^{14}C were at least two orders of magnitude smaller than those of ^{13}C, resulting in significant differences in net transfer between the labelling schemes, 14B-13F and 13B-14F ($P < 0·01$, data not shown). Significant effects of the labelling schemes were removed by applying a correction factor (CF) to excess $mg^{14}C$ on a treatment–species–tissue basis. The correction factor 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 severing treatment. For example, excess $mg^{13}C$ received by Douglas-fir fine roots measured in the labelling scheme, 13PB-14DF, was divided by excess $mg^{14}C$ received by Douglas-fir fine roots measured in the reciprocal labelling scheme, 14PB-13DF, of the same severing treatment. The treatment–species–tissue-specific CF values were averaged over the three replicates per labelling scheme. The corrected excess $mg^{14}C$ values (excess $mg^{14}C \times CF$) were analogous to excess $mg^{13}C$-equivalent values. Using the corrected excess $mg^{14}C$ values (i.e. ^{13}C-equivalent values), data were subjected to *t*-tests for pairwise comparisons of bidirectional and net transfer between unsevered and severed treatments ($n = 6$). Within severing treatments, net transfer was compared with zero using *t*-tests ($n = 6$).

RESULTS

Seedling characteristics

Paper-birch seedlings in the microcosms were twice as large as those of neighbouring Douglas fir ($P < 0·01$, Table 1). Total root biomass of paper birch was 2·2 times that of Douglas fir ($P < 0·01$), which was reflected in a larger root : shoot ratio ($P < 0·10$). Leaf net photosynthetic rate (PAR = 1000 μmol m^{-2} s^{-1}) of individual paper-birch seedlings was over twice that of Douglas fir ($P < 0·01$). Paper-birch leaves had greater specific leaf area than did those of Douglas fir because of their larger surface area and thinner leaf structure ($P < 0·01$). Leaf net photosynthetic rate, specific leaf area, and foliage biomass were used to calculate leaf area and net photosynthetic rate per seedling, which were 5 and 10 times greater, respectively, for paper birch than Douglas fir ($P < 0·01$). Foliar N concentration of individual paper-birch seedlings was almost double that of Douglas fir ($P < 0·01$).

Isotopic composition of paper birch and Douglas fir

Donors. Pulse-labelling resulted in mean ^{14}C and ^{13}C contents of 2·67 μg and 3·37 mg per donor seedling, respectively, with no differences between species or severing treatments (Table 2). This represented average pulse-labelling efficiencies of 20·2 % for ^{14}C and 11·5 % for ^{13}C. Douglas-fir and paper-birch seedlings pulsed-labelled with ^{14}C retained most of the isotope in their foliage and stems ($P < 0·01$, Fig. 2*b*). Pulse-labelled paper-birch roots contained 6 % of whole seedling ^{14}C content and Douglas-fir roots contained 30 %. For seedlings pulse-labelled with ^{14}C, isotope was more favourably distributed for below-ground export from Douglas fir than from paper birch. By contrast, seedlings pulsed with ^{13}C translocated over 70 % to roots, of which 85–90 % occurred in fine roots at the end of the chase period ($P = 0·04$ for Douglas fir, $P = 0·19$ for paper birch,

Table 2. *Total isotope content of paper-birch and Douglas-fir seedlings in the reciprocal transfer experiment, where seedlings were pulse-labelled with ^{13}C or ^{14}C and then harvested for isotope content after 6 d*

	Isotope content of donor seedlings		Isotope content of receiver seedlings	
Species	^{13}C (mg)	^{14}C (μg)	^{13}C (mg)	^{14}C (μg)
Paper birch	3·66 ± 1·47*	2·99 ± 0·11	1·75 ± 0·39	0·10 ± 0·04
Douglas fir	3·08 ± 1·04	2·36 ± 0·59	2·91 ± 0·52	0·15 ± 0·09

* Mean ± 1 SE.
Isotope differences between species were not significant ($P > 0·05$).

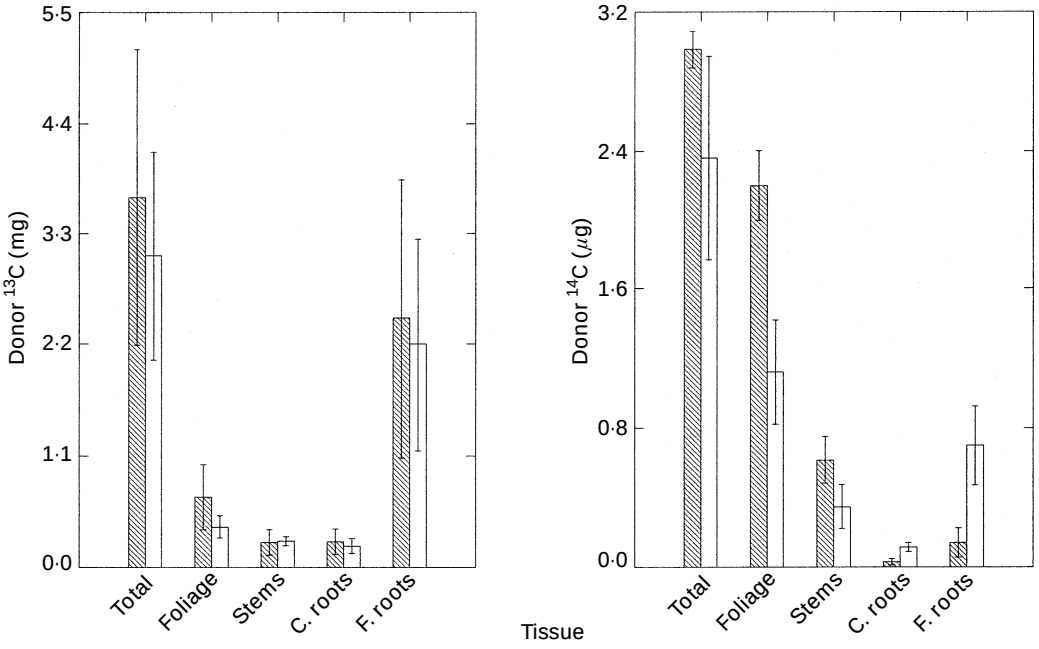

Figure 2. Total and tissue isotope content of donor paper-birch (▨) and Douglas-fir (□) seedlings pulse-labelled with (*a*) ^{13}C and (*b*) ^{14}C. Error bars are 1 SE; $n = 6$.

Fig. 2*a*). 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. Isotope fractionations reported by O'Leary (1981) and Craig (1954) are too small to account for the different labelling patterns between ^{13}C and ^{14}C. In our study, we consider ^{14}C the more reliable tracer of ^{12}C than ^{13}C.

Receivers. Both Douglas fir and paper birch received isotope from their neighbours, indicating that C transfer between paper birch and Douglas fir was bidirectional (Table 2). The amount of ^{14}C received by paper birch represented on average 3·3 % of its own isotope fixation by photosynthesis. Similarly, the amount transferred to Douglas fir represented 6·4 % of its own isotope fixation. Although Douglas fir received 50 % more ^{14}C ($P = 0·66$) and 66 % more ^{13}C ($P = 0·12$) from paper birch than vice versa, the differences in received isotope between species were not significant. These results indicate that transfer to Douglas fir was balanced by reciprocal transfer to paper birch (i.e. zero net transfer).

The distribution of ^{14}C and ^{13}C among tissues of receiver seedlings was similar between species. Transferred ^{14}C did not differ significantly among tissues of either species ($P = 0·47$ for paper birch, $P = 0·50$ for Douglas fir, Fig. 3*b*), mainly because of high variance in Douglas-fir stems and paper-birch foliage. The amount of ^{14}C that reached paper-birch and Douglas-fir foliage averaged 30 % of the total received. Similarly, transferred ^{13}C was evenly distributed among all tissues within paper birch ($P = 0·68$), but most remained in the roots of Douglas fir ($P < 0·01$, Fig. 3*a*). In both species, over 20 % of received ^{13}C occurred in foliage. These results indicate that ^{14}C and ^{13}C were readily translocated to all tissues of receiver seedlings.

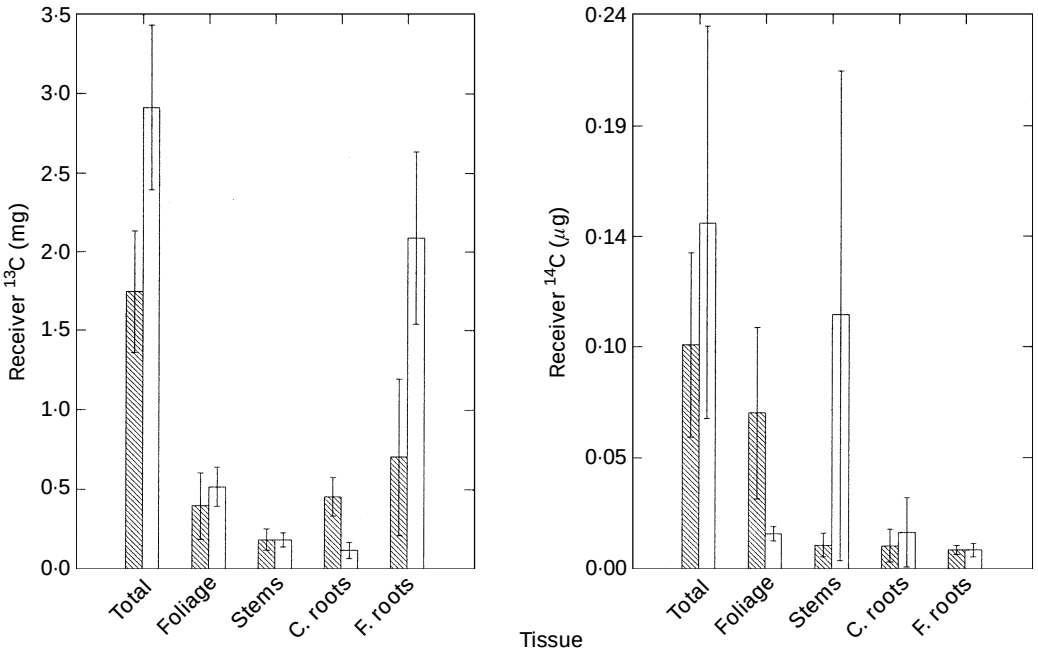

Figure 3. Tissue isotope content of receiver paper-birch (▨) and Douglas-fir (□) seedlings that received (*a*) ^{13}C and (*b*) ^{14}C. Error bars are 1 SE; $n = 6$.

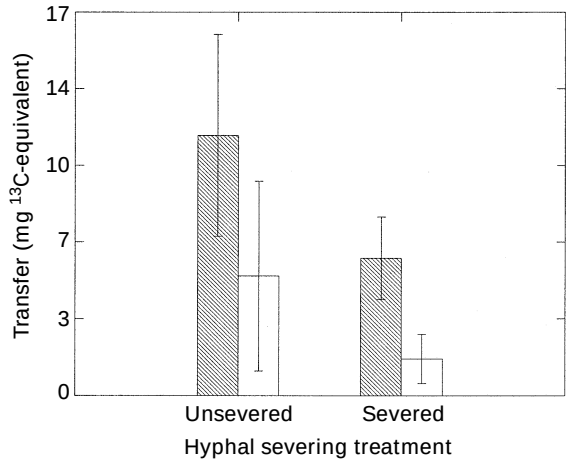

Figure 4. Bidirectional (▨) and net (□) transfer between paper birch and Douglas fir where interconnecting hyphae were intact and severed. Total amount of isotope transferred is expressed as mg ^{13}C-equivalent. Error bars are 1 SE; $n = 6$.

Effect of hyphae severing on bidirectional and net transfer

Bidirectional C transfer between paper birch and Douglas fir, whether calculated using ^{14}C alone or ^{14}C and ^{13}C together, did not differ significantly between severing treatments ($P > 0{\cdot}05$, Table 3, Fig. 4). Assuming that ^{14}C is the most reliable tracer of ^{12}C in this study, we estimate that bidirectional C transfer represented on average 5 % of total ^{14}C assimilated by paper birch and Douglas fir together. Severing had no effect on the amount of isotope received by Douglas fir from paper birch, or vice versa ($P > 0{\cdot}05$, Table 3).

Net transfer was positive in both hyphal connection treatments, suggesting a tendency for Douglas fir to receive more isotope than paper birch (Fig. 4). However, net transfer did not differ from zero in either treatment ($P > 0{\cdot}05$). Net transfer was approx. 3 times greater where hyphae were left intact than where they were severed; however, because of high variance, the difference between severing treatments was not significant ($P = 0{\cdot}59$).

DISCUSSION

Bidirectional C transfer

Transfer of C isotopes between paper birch and Douglas fir was bidirectional, supporting Newman's (1988) assertion that results of one-way labelling studies do not necessarily prove net movement to the receiver plant. This, to our knowledge, is the first report of reciprocal C transfer using dual isotope labelling within pairs of plants. Although both ^{14}C and ^{13}C were used to detect bidirectional transfer within microcosms, ^{14}C alone was used to estimate the magnitude of bidirectional ^{12}C transfer because the large $^{13}CO_2$ pulse (*c.* 1·4 % concentration) affected ^{13}C tissue allocation patterns. The large $^{13}CO_2$ pulse resulted in 70 % ^{13}C compared with only 18 % ^{14}C allocation to donor roots, which differs substantially from results of an associated field study, where out-planted paper birch and Douglas fir pulsed with similar $^{12+13}CO_2$ (*c.* 0·05 %) and $^{12+14}CO_2$ (*c.* 0·03 %) concentrations allocated 16 % each of ^{13}C and ^{14}C to roots. The small difference in $^{12+13}CO_2$ and $^{12+14}CO_2$ concentrations in Simard (1995) had no effect on isotope tissue-allocation patterns; this is

Table 3. *Carbon isotope received by paper birch and Douglas fir in the reciprocal transfer experiment, where hyphal connections were intact or severed*

Hyphae severing treatment	Received by Douglas fir		Received by paper birch		Bidirectional transfer	
	^{13}C (mg)	^{14}C (μg)	^{13}C (mg)	^{14}C (μg)	^{13}C (mg)	^{14}C (μg)
Intact	3·48 ± 0·63*	0·24 ± 0·21	1·75 ± 0·18	0·07 ± 0·02	5·24 ± 1·14	0·31 ± 0·09
Severed	2·34 ± 0·34	0·07 ± 0·03	1·74 ± 0·47	0·13 ± 0·09	4·09 ± 0·96	0·21 ± 0·04

* Mean ± 1 SE.
Isotope differences between severing treatments were not significant ($P > 0·05$).

supported by Simard *et al.* (1997*b*), who found no effect of increasing $^{12+13}CO_2$ concentrations from 0·04 % to 0·05 % on ^{13}C allocation patterns.

Based on ^{14}C alone, bidirectional transfer between paper birch and Douglas fir in our microcoms represented on average 5 % of total ^{14}C assimilated by both species together. Results were similar to those from Simard's (1995) field study, where bidirectional C transfer between outplanted paper-birch and Douglas-fir seedlings represented on average 4–7 % of total isotope assimilated by donors. This comparison suggests that the laboratory experiment provided a realistic estimate of bidirectional transfer in plant communities in the field. Both studies might underestimate somewhat bidirectional ^{12}C transfer, however, given that short pulses of C isotopes do not necessarily result in an even distribution of isotope among all organic compounds that might be transferred (e.g. Jenkinson, 1971; Warembourg & Paul, 1973).

In the present study, the amount of ^{14}C transferred to Douglas fir alone represented on average 6·4 % of that which it fixed itself by photosynthesis, and the amount transferred to paper birch alone represented 3·3 % of its own photosynthesis. Conversely, one-way transfer to Douglas fir or paper birch represented 5·0 % and 4·2 % of isotope fixation by donor paper birch and Douglas fir, respectively. These results suggest that Douglas fir potentially has more to gain than does paper birch from interspecifc C transfer. The results are similar to those of a pilot study using ^{13}C alone, where one-way belowground transfer from paper birch to Douglas fir represented 4·4 % of isotope fixation by donor birch (Simard *et al.* 1997*b*).

The amounts of isotope transferred from paper birch to Douglas fir and vice versa exceed one-way mycorrhizal-mediated ^{14}C transfer measured in previous laboratory studies (Hirrel & Gerdemann, 1979; Francis & Read, 1984; Read *et al.*, 1985; Finlay & Read, 1986; Waters & Borowicz, 1994), where ^{14}C found in receiver plants usually represented 1 % or less of that found in interconnected donor plants. One possible explanation is that hyphal connections might have been formed by more than one fungal species in this study, possibly providing multiple transfer pathways, whereas in previous studies 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' (Miller & Allen, 1992).

Whether C was translocated directly through EM fungi or indirectly through soil pathways remains in doubt for two reasons. Firstly, the presence and functional status of hyphal connections between paper birch and Douglas fir were not rigorously tested using autoradiography, as was done by Francis & Read (1984). Hyphal connections probably occurred, however, because (i) paper birch and Douglas fir shared seven EM morphotypes over 90 % of their root tips in a bioassay using the same plant and soil material as this study (Simard *et al.*, 1997*a*), and (ii) multiple hyphal connections between root zones of paper birch and Douglas fir were visible to the naked eye before labelling. Secondly, differences in C transfer between unsevered (i.e. through EM connections plus soil pathways) and severed microcosms (i.e. through soil pathways alone) were not statistically significant. Bidirectional transfer averaged 40 % greater and net transfer was over 3 times greater where hyphae were left intact than where they were severed immediately before pulse labelling; however, these differences did not approach statistical significance because of high variability among replicates. These results suggest that hyphae might facilitate interspecific C-transfer, but it is a highly variable process that requires considerably more replication to detect than we used in our experiment. Alternatively, some severed hyphae might have anastomosed and partly reconnected paper-birch and Douglas-fir roots during the 6-d chase period. This is possible, given that growth rates of *Thelephora terrestris* and *Laccaria proxima* mycelial fans originating from *Picea sitchensis* roots have been measured at 1–4 mm d^{-1} in field soils in mid-summer, rates which are comparable to those in glasshouse conditions (Coutts & Nicoll, 1990). Finlay & Read (1986) also measured rates of mycelial spread and strand extension of *Suillus bovinus* and *Pisolithus tinctorius* from *Pinus contorta* seedlings at 9–16 cm^2 d^{-1} and 2–4 mm d^{-1},

respectively. Given that the root pouches were in close contact, results from these previous studies suggest that radiating hyphae from each seedling could have at least partly re-formed interconnecting strands during the 6-d chase.

Alternative pathways for C transfer in our study include (i) fungal and root exudate, (ii) CO_2 respired from roots, (iii) CO_2 respired from shoots, or (iv) sloughed fungal and root cells. Our evaluation of these pathways is as follows. (i) The portion of photosynthate exuded into the rhizosphere has been estimated in some studies as small, ranging between $< 1\%$ and 4% (Paul & Kucey, 1981; Miller, Durall & Rygiewicz, 1989; Jakobson & Rosendahl, 1990), and in other studies as much higher, ranging between 10% and 40% (Whipps & Lynch, 1986; Reid & Mexal, 1977). The proportion of ^{14}C transferred between paper birch and Douglas fir in this study falls within the range of published exudate estimates. It is possible that C isotope was exuded into the rhizosphere soil by mycorrhiza of donor seedlings, and then was picked up by mycorrhiza of neighbouring receiver seedlings. (ii) Fungal and root respiration have been shown to account for up to 33% of photosynthate in other studies (Paul & Kucey, 1981; Harris, Pacovsky & Paul, 1985). However, in a pilot study using seedlings grown under similar conditions as the present experiment, anaplerotic uptake of respired CO_2 by roots of paper-birch and Douglas-fir seedlings was not detected (Simard, 1995). Consequently, we discount anaplerotic uptake as a significant transfer route in this study. In addition, re-fixation of root-respired CO_2 by foliage of neighbouring seedlings was not considered important because roots remained sealed off from shoots during the pulse and chase periods. (iii) It is possible during bag removal and the chase period inside the fume-hood that some isotope was released or respired by donor seedlings, and subsequently re-fixed by receiver foliage through photosynthesis. However, in a pilot study that examined one-way ^{13}C transfer from paper birch to Douglas fir using the same microcosms and labelling methodology as the present study, Simard *et al.* (1997*b*) found that only $0{\cdot}31\%$ of ^{13}C fixed by donor paper birch was released or respired and then re-assimilated by foliage of Douglas fir in control microcosms during a 6-d chase period. This above-ground uptake by control Douglas fir was minor compared with the $4{\cdot}4\%$ transferred below-ground from donor paper birch to receiver Douglas fir. Consequently, we consider foliar uptake by receiver seedlings of isotope released into the fumehood during the chase as a minor transfer pathway in this study. (iv) Little is known about rates of C input to the soil pool through death and decomposition of mycorrhizal hyphae (Finlay & Söderström, 1992). The short chase-period in the present study, however, likely resulted in little isotope input due to decomposition of sloughed material. Based on this information, we assume that the most important soil pathway for indirect C transfer was root and fungal exudates.

Net C transfer

Douglas fir received on average 50% more ^{14}C and 66% more ^{13}C from paper birch than vice versa, but the differences between species were not statistically significant. Net-transfer estimates based on both ^{13}C and corrected ^{14}C values were positive on average, suggesting a tendency for Douglas fir to receive more isotope from paper birch than vice versa. However, net-transfer estimates did not differ significantly from zero, either where hyphae were intact or where they were severed before labelling. Net transfer was over 3 times greater where hyphae were left intact than where they were severed, but differences between severing treatments were not significant. As with bidirectional transfer, these results suggest that interconnecting hyphae might have played some role in facilitating transfer between paper birch and Douglas fir, but that the relative importance of hyphal connections vs. soil pathways is unclear.

The microcosm results differed from the field experiments of Simard (1995), where net transfer occurred from paper-birch to Douglas-fir seedlings that were outplanted into the same forest soil used in the present microcosms. When seedlings had been outplanted in the field for 2 yr, net C gain by Douglas fir represented on average 6% of C isotope uptake by photosynthesis. Furthermore, lowering the net photosynthetic rate of Douglas fir by shading significantly increased net transfer to Douglas fir. This contrasted with the previous year, when transfer to Douglas fir was balanced by reciprocal transfer to paper birch, regardless of shading. In both years, neighbouring AM *Thuja plicata* seedlings absorbed minor amounts of isotope, suggesting that C transfer between paper birch and Douglas fir occurred primarily through interconnecting EM hyphae. The increase in net transfer as seedlings aged was associated with greater root extension and potential for hyphal linkages, as well as increased seedling vigour, indicating that transfer varies depending on seedling status and environmental conditions. There are several possible explanations for the differences in net-transfer estimates between our microcosm experiment and the field experiments of Simard (1995), including a lower degree of hyphal connection, greater vigour of Douglas-fir relative to paper-birch seedlings, different spatial patterns in available nutrients, and lower replication in the microcosm than the field experiments.

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 photosynthetic rate, nutrient status, or capability of fixing atmospheric N_2 (e.g. Read *et al.*,

1985; Newman, 1988; Alpert, Waremberg & Roy, 1991; Bethlenfalvay *et al.*, 1991; Arnebrant *et al.*, 1993). In the present study, whole seedling net photosynthetic rate of paper birch was estimated to be 10 times that of neighbouring Douglas fir both during labelling (PAR = 1000 μmol m^{-2} s^{-1}) and the chase (PAR = 300–400 μmol $m^{-2} < s^{-1}$, data not shown). In general, leaves of deciduous tree species have the potential for faster photosynthetic rates than conifers, as a result of differences in their diffusion pathway for CO_2 (Waring & Schlesinger, 1985). Net photosynthesis values for paper birch fell within the range reported by Wang, Simard & Kimmins (1995) and Ranney, Bir & Skroch (1991), and those for Douglas fir were similar to rates reported by Dosskey, Linderman & Boersma (1990). Paper-birch foliar N concentration also was approx. twice that of Douglas fir, and foliar N concentration has a well-known relationship with net photosynthetic rates (Brix, 1981; Pearcy *et al.*, 1987; Wang *et al.*, 1995). Further, Simard *et al.* (1997*b*) found that paper-birch seedlings allocated a greater proportion and amount of current photosynthate to fine roots than did Douglas-fir seedlings, which they suggest might result in greater potential for C transfer from paper birch to neighbouring plants. In this study, paper-birch biomass was double and root : shoot ratio 1·23 times that of neighbouring Douglas fir, but whether plant size differences alone can influence source–sink relations has been questioned (Newman, 1988). The combined differences in net photosynthetic rate, foliar N concentration and C allocation patterns between paper birch and Douglas fir may have contributed to the tendency for Douglas fir to receive more isotope than paper birch in our microcosms, and might provide a source–sink mechanism for net transfer from paper birch to Douglas fir in the field experiment of Simard (1995). However, the variability in transfer in our microcosm experiment may suggest a more complex transfer mechanism than that governed by the relative sink strength of host seedlings alone, and could also include the nutritional demands of interconnecting fungi as well as availability of soil nutrients.

Whether bidirectional C transfer between paper birch and Douglas fir is of significance to seedling performance or fitness, or how the seedlings interact over a longer time period, cannot be determined from our study. Furthermore, because net-transfer did not occur, there is no evidence that one species can benefit from the association more than the other. However, other experiments indicate that net transfer can occur between paper birch and Douglas fir under some conditions. Simard's (1995) estimate of 6 % net transfer from paper birch to Douglas fir was similar to the 10 % ^{14}C transferred from clonal *Eichhornia crassipes* parents to offspring ramets (Alpert *et al.*, 1991), which the authors suggest to be of sufficient magnitude to affect survival and growth of the ramets. Carbon transfer of this magnitude, whether momentary or prolonged, could affect seedling performance under particularly stressful conditions or over the lifetime of a seedling. For example, C transfer from paper birch to Douglas fir could be important to Douglas-fir survival under conditions where its photosynthetic potential is low, such as in deep shade, drought or cold temperatures. Conversely, paper birch may benefit from the association with Douglas fir if the direction of transfer is reversed during early spring or fall when paper-birch foliage is absent or expanding.

Distribution of transferred C in receiver tissues

The distribution of transferred isotope in receiver seedling tissues varied in response to whether ^{14}C or ^{13}C was transferred. The large increase in CO_2 concentration caused by the ^{13}C pulse resulted in greater ^{13}C (70 %) than ^{14}C (18 %) allocation to roots of donor plants in our study. Consequently, a greater proportion might also have been available for interplant transfer, which potentially could affect isotope distribution in receiver plants. To that end, we consider ^{14}C the more reliable tracer of the fate of received ^{12}C.

Transferred ^{14}C was evenly distributed among all tissues of receiver paper birch and Douglas fir, mainly because of the high variance in Douglas-fir stems and paper birch foliage. Substantially more ^{14}C was translocated on average into foliage of receiver paper birch and Douglas fir (30 %) than has been observed in previous EM carbon transfer experiments (usually < 10 %, Newman 1988). Carbon transfer in our EM system also contrasts with that in most AM systems, where none or little of the transferred C was translocated into shoots of receiver plants, leaving open the possibility that transferred C remains in the fungal tissues (e.g. Francis & Read, 1985; Waters & Borowicz, 1994; Watkins *et al.*, 1996). This transferred isotope in foliage of paper birch and Douglas fir could either supplement C in photosynthate or foliar N by functioning as the C skeleton in amino acids. Translocation of ^{14}C from receiver roots to foliage might occur along an N rather than C concentration gradient because fully developed leaves are usually strong sinks for N and are sources, rather than sinks, for C (Pearcy *et al.*, 1987).

CONCLUSIONS

The large proportion of assimilated isotope that was exchanged below-ground between paper birch and Douglas fir in our study is indicative of a tightly-linked seedling–soil system (Perry *et al.*, 1989). Because there was no net transfer between species, however, the net effect of below-ground transfer on seedling performance or species interactions is unclear. These results show that C transfer is a

highly variable process, which might be affected by source–sink gradients between plants, as well as by root, fungal and soil factors. Whether transfer occurs through interconnecting hyphae, soil pathways, or both, can only be resolved through further research. This experiment could be repeated, for example, with greater replication, better hyphae severing procedures, a lower $^{13}CO_2$ pulse, and different statistical techniques. Further research is required to evaluate the implications of C transfer to plant performance, below- and above-ground community dynamics, and species diversity in the field.

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