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^{14}C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings via arbuscular mycorrhizal fungi in natural stands

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Abstract We investigated in the field the carbon (C) transfer between sugar maple (*Acer saccharum*) saplings and the spring ephemeral *Erythronium americanum* via the mycelium of arbuscular mycorrhizal (AM) fungi. Sugar maple saplings and *E. americanum* plants were planted together in pots placed in the ground of a maple forest in 1999. Ectomycorrhizal yellow birches (*Betula alleghaniensis*) were added as control plants. In spring 2000, during leaf expansion of sugar maple saplings, the leaves of *E. americanum* were labelled with $^{14}CO_2$. Seven days after labelling, radioactivity was detected in leaves, stem and roots of sugar maples. Specific radioactivity in sugar maples was 13-fold higher than in yellow birches revealing the occurrence of a direct transfer of ^{14}C between the AM plants. The quantity of ^{14}C transferred to sugar maple saplings was negatively correlated with the percentage of ^{14}C allocated to the storage organ of *E. americanum*. A second labelling was performed in autumn 2000 on sugar maple leaves during annual growth of *E. americanum* roots. Radioactivity was detected in 7 of 22 *E. americanum* root systems and absent in yellow birches. These results suggest that AM fungi connecting different understorey species can act as reciprocal C transfer bridges between plant species in relation with the phenology of the plants involved.

Keywords Carbon transfer · Arbuscular mycorrhiza · Sugar maple · *Acer saccharum* · *Erythronium americanum*

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Introduction

Mycorrhizal fungi are symbiotic partners associated with the great majority of land plant species (Smith and Read 1997). They colonise roots and improve plant nutrition mainly by transferring phosphate (P) from the soil to the plant. The plants provide the fungi with carbohydrates (Smith and Read 1997). Because of low host specificity of mycorrhizal fungi their mycelium can form a network that interconnects host plants of the same (Brownlee et al. 1983; Newman and Eason 1993; Graves et al. 1997) or different species (Francis and Read 1984; Arnebrant et al. 1993). This means fungal hyphae can provide pathways to transfer of compounds between mycorrhizal plants. Isotope tracer studies have documented transfer of water (Duddridge et al. 1980) and nutrients such as P (Newman and Eason 1993), nitrogen (N) (Arnebrant et al. 1993) and carbon (C) (Francis and Read 1984; Finlay and Read 1986).

The occurrence of direct C transfer from donor to receiver plants through fungal mycelium has been mostly studied under laboratory and greenhouse conditions (Francis and Read 1984; Finlay and Read 1986; Simard et al. 1997a) with arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi. On most of these studies receiver plants were artificially shaded demonstrating that C transfer is strongly governed by a source-sink relationship. Nevertheless, physiological importance of such transport in ecosystems has not been proven (Robinson and Fitter 1999). Brownlee et al. (1983) hypothesised that newly germinated seedlings of *Pinus sylvestris* could receive photoassimilates from mature trees in their vicinity and overcome forest understorey shade conditions. Recently, Simard et al. (1997b) measured net transfer of C in the field between seedlings of two EM tree species *Betula papyrifera* and *Pseudotsuga menziesii*. There was a much smaller C transfer (18% of that between the EM species) towards AM tree seedlings of *Thuja plicata* present in the vicinity. They also found that the amount of C exported from the donor plant was positively correlated with the level of shading on the receiver plant. To our knowledge, C transfer between spe-

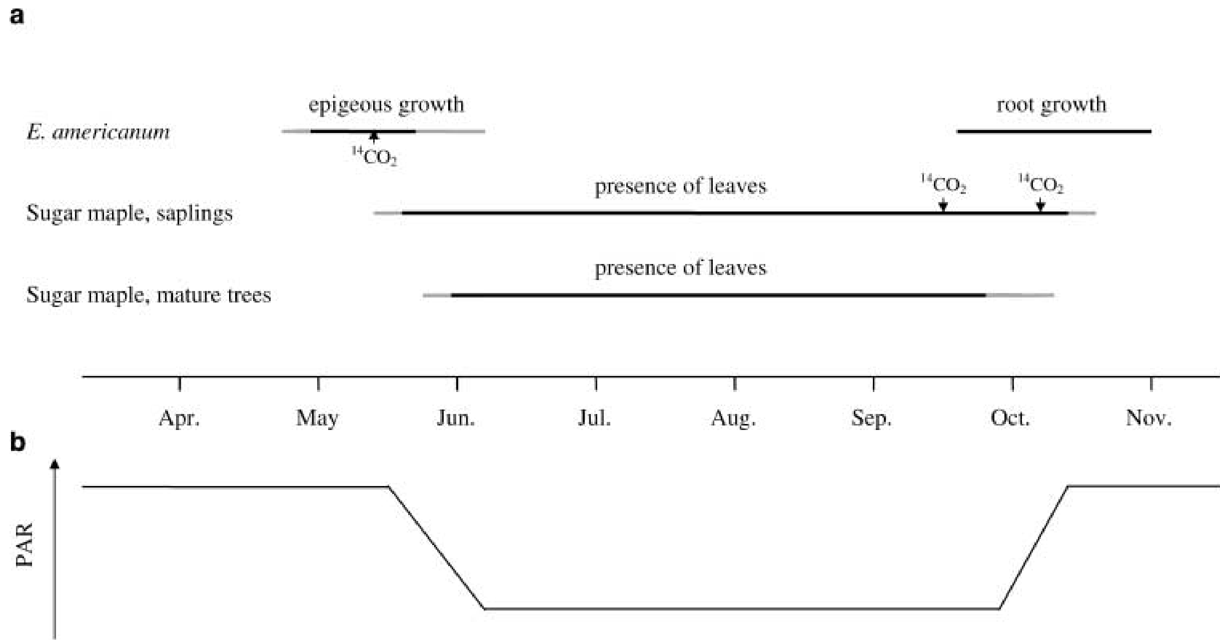

Fig. 1 Phenology of *Erythronium americanum* and leaves of sugar maple saplings and mature trees (**a**) and relative PAR (photosynthetically active radiation) on the ground of a maple forest (**b**) over a growing season in the region of Québec City. *Grey bars* represent development (beginning of line) or senescence (end of line) of leaves. Dates of labelling with $^{14}CO_2$ are represented by *arrows*

cies with different developmental phenology has not been reported within a plant community.

In natural plant communities dominated by endomycorrhizal species, AM fungi rapidly colonise roots of newly germinated seedlings and plants that renew their entire root system every year (for example Liliaceous spring ephemerals). Spores of AM fungi are an improbable inoculum because of their low viability in the soil (Read et al. 1976; Zahka et al. 1995). Therefore, the most likely source of inoculation of new roots is hyphae from the roots of neighbouring plants previously colonised by AM fungi.

Erythronium americanum Ker-Gawl (trout lily) is an abundant spring ephemeral in maple forests of North America that is colonised by AM fungi (Brundrett and Kendrick 1990b; Lapointe and Molard 1997). In the region of Québec City, epigeous development of *E. americanum* follows snow melt in late-April when the tree canopy is completely open (Fig. 1a, b). *E. americanum* takes advantage of these full light conditions for active carbohydrate assimilation until canopy closure. Carbohydrate storage in the corm (an underground reserve organ) is completed 3 weeks after leaf expansion but high levels of photosynthesis (10–14 μmol CO_2 m^{-2} s^{-1}) are maintained until the overstorey canopy starts to close (Taylor and Pearcy 1976; Lapointe, unpublished data). Leaves of *E. americanum* then senesce during canopy closure. Leaf senescence is completed by mid-June followed by root senescence at the beginning of July (data not shown). In early autumn (mid-September) *E. americanum* produces new unbranched roots that are rapidly colonised by AM fungi (Brundrett and Kendrick 1990a; Lapointe and Molard 1997).

Sugar maple (*Acer saccharum* Marsh) is a common North American tree species associated with AM fungi (Yawney and Schultz 1990; Cooke et al. 1993). Bud burst of sugar maple saplings occurs about 3 weeks after leaves of *E. americanum* have unfolded and started to photosynthesise whilst bud burst of mature sugar maple trees is delayed by 2 weeks (Fig. 1a). Thus, the expansion of the leaves of sugar maple saplings occurs under high light conditions. Leaf development is a costly process in terms of carbohydrates (Poorter and Villar 1997) and sugar maple saplings could receive C during leaf expansion through mycorrhizal hyphae from neighbouring plants already photosynthetically active.

In the autumn, mycorrhizal colonisation of *E. americanum* roots occurs during root growth, which represents a significant carbohydrate cost at a time when plants do not have leaves (Lapointe and Molard 1997). C required for root production in *E. americanum* could be partly supplied by photosynthetically active neighbouring sugar maple trees and juveniles via interconnecting mycelium.

The goal of this work was to test for reciprocal and temporal transfers of C between the spring ephemeral *E. americanum* and 1-year-old saplings of the woody species sugar maple in a natural plant community. Such net transfers would be restricted in time to the period when one species is photosynthetically active while the other is not.

Materials and methods

Study site

The experiments were carried out in a sugar maple forest in Saint-Augustin-de-Desmaures (46°48′ N, 71°23′ W) in the vicinity of Québec City. The site has been previously described in Lapointe and Molard (1997). The canopy is dominated by *Acer saccharum* Marsh with *A. rubrum* L., *Fraxinus americana* L. and *Ulmus rubra* Mühl as companion species. The soil is a clay-loam with a

thin moder type humic horizon. The most abundant understorey species are *Erythronium americanum* Ker-Gawl, *Trillium erectum* L. and *Veratrum viride* Ait.

Experimental design

In spring 1999 (mid-May), 60 organic fibre pots (v=3 l, d=16 cm, h=16 cm) were buried into the soil at the study site to a depth allowing the edge to remain 1–2 cm over ground level. Pots were filled with natural soil collected at the site. The soil included fine roots of mature trees as a source of mycorrhizal inoculation for sugar maple seedlings. One-year-old seedlings of sugar maple were carefully collected near the experimental site and transplanted into the middle of each pot (one seedling per pot). Five weeks later, three corms of *E. americanum* were planted 5 cm deep into each pot. At that time, leaves of the transplanted *E. americanum* had already senesced and corms had entered summer dormancy. In August, yellow birch saplings (*Betula alleghaniensis* Britton) (<12-cm high) were collected from a distant site at l'Île-aux-Grues (47°03′ N, 75°28′ W). Birches are typically colonised by EM fungi rather that AM fungi. Presence of EM features was visually checked and seedlings with the highest mycorrhization were transplanted into the pots (one per pot). Pots were regularly removed from soil and immediately replaced to avoid possible intrusion of roots from surrounding trees.

Labelling experiments with ^{14}C

Spring labelling

In mid-May 2000, at bud burst of sugar maple and yellow birch saplings (Fig. 1a), 12 of the pots were randomly selected for ^{14}C labelling. The leaf of one of the three *E. americanum* plants in the selected pots was enclosed in a 3.7-l transparent freezer bag (Ziploc) that was firmly anchored between two wooden stakes. A 12-cm-rubber tube (d=5 mm) was fixed to an upper corner of the bag and tightly closed during the experiment. A sealing compound placed around the stem of the plant insured hermetic closure of the bag during labelling. A 29.5-ml cup containing a basic solution of 185 kBq (5 µCi) $NaH^{14}CO_3$ (Amersham Pharmacia Biotech) was placed inside the plastic bag and the bag tightly closed. Pulse labelling started when 1 ml lactic acid (85%) was syringe-injected through the bag into the cup to liberate gaseous $^{14}CO_2$ (the release of $^{14}CO_2$ in the bag was estimated to increase the CO_2 concentration by 4–6 ppm). Plants were left exposed to sunlight (>1,000 µmol m^{-2} s^{-1} PAR, photosynthetically active radiation) for 1.5 h. At the end of the experiment, excess $^{14}CO_2$ was evacuated through the rubber tube and trapped in a 1 *N* NaOH solution.

After a 7-day chase period, which allowed complete expansion of sugar maple leaves, all plants in the pots were harvested and dried at 65°C for 24 h except for roots of non-labelled *E. americanum* plants, which were collected for determination of mycorrhizal colonisation.

Autumn labelling

In the autumn 2000, two consecutive labelling sessions were performed (Fig. 1a). In mid-September, 12 pots were chosen for labelling sugar maples. At this time in early autumn, leaves of sugar maple saplings were still green and capable of photosynthesis (assimilation rate: 1.7±0.8 µmol CO_2 m^{-2} s^{-1}) while *E. americanum* corms had no functional roots. This early session insured labelling of the sugar maple saplings before leaf senescence. The technical procedure was identical to that used in spring with the following modifications. Pots were moved to an open field where ambient sunlight reached 1,200 µmol m^{-2} s^{-1} PAR and the whole foliage of sugar maple 1-year saplings was pulse-labelled in plastic bags for 2 h. Three weeks later, root samples harvested in other pots showed that *E. americanum* corms had formed new roots and these had been colonised by AM fungi. At this time leaves of the previously labelled sugar maples were still green, and the plants were labelled a second time. Pots were harvested 2 weeks after the second labelling session (14-day chase period). Plant material was destructively harvested and dried with the exception of all *E. americanum* roots that were stored in a FAA solution.

Determination of radioactivity

Spring labelling

Dried sugar maple, non-labelled *E. americanum* (root excepted) and yellow birch were exposed to autoradiography film (Kodak BioMax). The films were developed after 6 weeks of exposure.

Leaves, stem and roots of sugar maple saplings and labelled *E. americanum* and leaves of non-labelled *E. americanum* were separated and ground in liquid nitrogen, likewise whole yellow birch saplings. An aliquot of each material type (15–50 mg) was digested as in Clifford et al. (1973) and radioactivity determined by liquid scintillation spectrometry. Counts were standardised with a quench curve and radioactivity was expressed in dpm.

Autumn labelling

Yellow birch saplings and dried corms of *E. americanum* were exposed to autoradiography film. The films were developed after 6 weeks of exposure.

Roots samples of *E. americanum* were fixed in paraffin and 5-µm thick sections were mounted on microscope slides. Paraffin was then dissolved with toluene and root sections were coated with LM-1 nuclear emulsion (Amersham Pharmacia Biotech; crystal size of 0.2 µm). The emulsion was processed after exposure at 4°C for 2–4 weeks. The rest of *E. americanum* root samples were rinsed several times with distilled water, dried, weighed and assessed for radioactivity as described previously using liquid scintillation. Radioactivity in yellow birch saplings was also assessed.

Mycorrhizal colonisation levels

Roots of *E. americanum* were cleared in a 10% (w/v) KOH solution for 12 min at 90°C prior to staining with a trypan blue solution (Koske and Gemma 1989). AM colonisation was assessed microscopically at ×78 magnification using the method described by Trouvelot et al. (1986). The following parameters were calculated: frequency of colonisation, intensity of colonisation and arbuscular content.

Statistical analysis

One-way ANOVA was performed to compare radioactivity data in sugar maple and yellow birch saplings after spring labelling using the GLM procedure of the SAS statistical package.

Results

Following spring radiolabelling of *E. americanum* plants, the macro-autoradiography revealed traces of ^{14}C in all sugar maple saplings (Fig. 2a–c). In contrast, no labelling was observed in yellow birch saplings and non-labelled *E. americanum* growing in the same pots as the labelled *E. americanum* (autoradiographs not shown), indicating absence of transfer between *E. americanum* plants. Roots, stem or leaves of the sugar maple saplings

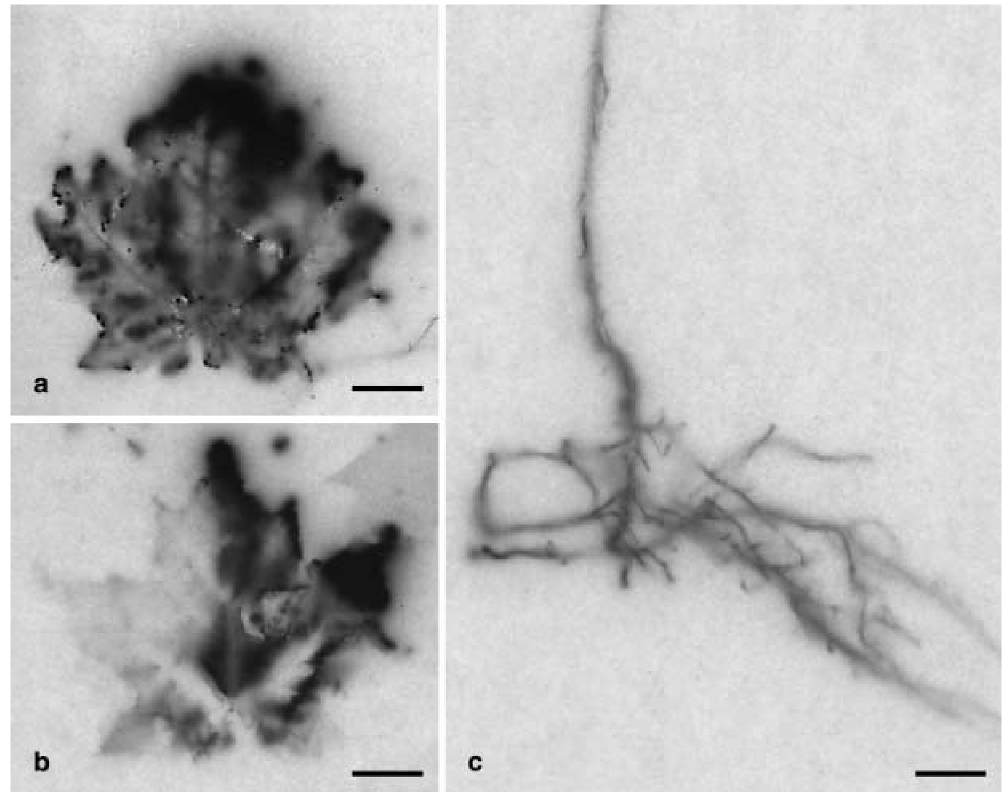

Fig. 2 Macro-autoradiographs of leaves (**a**, **b**) and stem and root system (**c**) of labelled C in receiver sugar maple saplings. Dry plant material was exposed for 6 weeks on Kodak scientific imaging films. *Bars* represent 1 cm

Table 1 Specific activity (dpm g^{-1} dry matter), biomass ratio, and distribution of radioactivity in leaves, stem, and roots of sugar maple saplings 1 week after spring labelling. Data represent means±1 SE

	Leaves	Stem	Roots
Specific activity	6,186±5,953	2,386±2,552	2,941±4,360
Biomass ratio	30.8±11.4%	36.6±13.4%	32.6±7.4%
Percent radioactivity	50.4±24.6%	27.6±16.3%	22.0±16.9%

were labelled with ^{14}C. Five of the saplings were extensively labelled in the leaves. Two of the saplings showed only slight labelling in their roots.

Total radioactivity (dpm) and specific radioactivity (dpm g^{-1} dry matter) were much greater in sugar maple compared to yellow birch saplings ($P<0.01$). Average total radioactivity in sugar maple saplings was 738±642 dpm (means±1 SE; range, 136–2,233 dpm) and was 15±26 dpm (range, 0–82 dpm) in yellow birch saplings. Average specific radioactivity was 3,884±3,046 dpm g^{-1} dry matter in sugar maple saplings and was 290±450 dpm g^{-1} dry matter in yellow birch saplings. Small amounts of radioactivity (224±318 dpm g^{-1} dry matter) were detected in the leaves of non-labelled *E. americanum* indicating that very little ^{14}C was absorbed by photosynthetically active leaves during the chase period. In sugar maple saplings specific activity was the highest in leaves (Table 1). Radioactivity was thus mainly localised in the shoot (leaves and stem), with less radioactivity measured in the roots (Table 1). In labelled *E. americanum* the ^{14}C content was $1.12\times10^6\pm0.22\times10^6$ dpm (18.6±3.6 kBq), which was equivalent to ca. 10% of the $^{14}CO_2$ used for labelling. On average, 53.9±30.4% of the total was found in the corm of labelled *E. americanum* plants. The total activity in the sugar maple saplings was 0.064±0.049% of that in *E. americanum* while dry biomass was 195±76 mg for sugar maple saplings and 292±96 mg for *E. americanum*. It appeared that when the percentage of ^{14}C in the corm of labelled *E. americanum* was the lowest, the ^{14}C content in sugar maple saplings was the highest. Consequently, a negative correlation ($r^2=0.61$, $P=0.0028$) was established between the percentage of ^{14}C translocated to the corm of labelled *E. americanum* and the percentage of ^{14}C translocated to sugar maple saplings (Fig. 3).

The root systems of non-labelled *E. americanum* plants showed high levels of root colonisation by AM fungi in spring. Frequency of colonisation was 44.7±27.0% ($n=28$), intensity of colonisation was 13.3±14.7% and arbuscular content was 84.8±19.6%.

In autumn, after sugar maple labelling, ^{14}C was detected with liquid scintillation in 7 of 22 *E. americanum* root samples. The average total radioactivity was 230±269 dpm (range, 43–645 dpm) and the average specific radioactivity was 4,244±5,288 dpm g^{-1} dry matter for the 7 labelled root systems. No radioactivity was detected in the yellow birch saplings. ^{14}C content in labelled sugar maple saplings was $1.12\times10^6\pm0.51\times10^6$ dpm (20.8±8.5 kBq), which was less than 6% of $^{14}CO_2$ used for labelling the plants. The total activity in the seven labelled *E. americanum* root systems was 0.018±0.021% of that in sugar maple saplings.

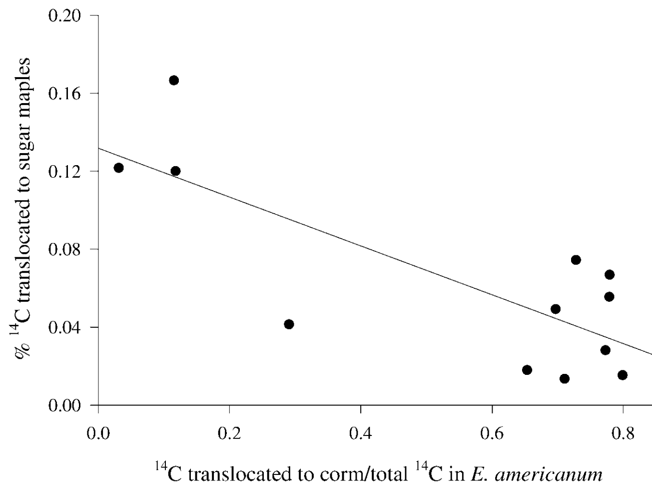

Fig. 3 Relationship between the percentages of ^{14}C exported to sugar maple saplings following spring labelling of *Erythronium americanum* and the fraction of ^{14}C in the corm of labelled *E. americanum*

Radioactivity was not detected on autoradiographs after 6 weeks of exposure in *E. americanum* corms, or in yellow birch saplings. Microscope slides of *E. americanum* roots showed no trace of radioactivity after 2–4 weeks of exposure to LM-1 nuclear emulsion. After 4 weeks, the background was too high to detect ^{14}C in the root and fungal tissues.

Discussion

This is the first report, to our knowledge, of source-sink relationships that were not governed by shading the receiver plants but by the phenology of receiver and donor plants.

In our experiment autoradiographs and quantitative analyses of radioactivity revealed ^{14}C in all sugar maple saplings. Sugar maple saplings showed labelling not only in roots but also in leaves and stem clearly demonstrating that (1) ^{14}C transfer from the AM fungi to the receiver plants occurred and (2) the transferred ^{14}C was translocated into the plant and thus available for plant growth. Earlier C transfer studies reported ^{14}C only in the roots of the receiver plants (Francis and Read 1984; Finlay and Read 1986; Wu et al. 2001). However, because ^{14}C could be confined to the AM fungal tissues colonising the roots, labelled C in the shoot is clear evidence for C transfer from a donor plant via the mycorrhizal mycelium to a receiver plant.

We found no visible radioactivity in yellow birches and non-labelled *E. americanum* on autoradiographs (data not shown) indicating that absorption of ^{14}C-root exudates by EM and AM fungi was insignificant. The low levels of ^{14}C detected in the quantitative analyses of ^{14}C of yellow birch showed that passive absorption of radiolabelled material could occur. However, average ^{14}C specific activity in sugar maple saplings was 13-fold higher than in yellow birch saplings while the lowest ^{14}C specific activities in sugar maples were comparable with yellow birches. It is likely that in the low activity sugar maples the ^{14}C detected in those saplings was due to passive absorption of labelled root exudates by associated AM fungi or the saplings roots.

The fraction of ^{14}C fixed (and retained) by the *E. americanum* and delivered to the sugar maples saplings (0.064±0.049%) seems tiny. However, we have estimated that over the 7 days of leaf expansion of sugar maple saplings with optimal sunlight conditions, an *E. americanum* plant with photosynthetic rates of 12 μmol CO^2 m^{-2} s^{-1}, 6 h per day, would transfer up to 21 μg C to sugar maples. From results of another study (unpublished data) where starch and sucrose contents were measured in sugar maple saplings in late autumn after bud formation and spring we have assessed the cost of leaf expansion in our sugar maple sapling was about 1.32 mg C per sapling. Thus, the C transferred from one *E. americanum* could cover up to 1.6% of leaf expansion of 1-year-old sugar maple saplings. It is likely that in undisturbed ecosystems sugar maple saplings would be connected to several *E. americanum* plants, as *E. americanum* is often more abundant than sugar maple saplings (personal observation). We can thus expect that sugar maple saplings would receive more C than measured in our one-to-one labelling experiment, and that the C contribution of *E. americanum* during sugar maple sapling leaf expansion is physiologically significant.

The distribution of radioactivity in sugar maple saplings was consistent with the concept of source-sink relationships in C transfers between donor and receiver plants. Seven days after the exposure of potential donor plants to ^{14}C, the label had been transferred to the sugar maple saplings and half of it was localised in leaves, which represented less than one-third of the total sapling biomass. This labelling pattern would be a consequence of bud burst in sugar maple saplings that occurred at the time the labelling experiment was performed. New leaves development acts as a strong sink for carbohydrates and thus directs high quantities of C towards these growing organs. Our results are consistent with those of Francis and Read (1984), Finlay and Read (1986) and Simard et al. (1997b) who underlined the essential role of source-sink relationships between plants connected by common mycelia. C transfer between plants was modified by applying different levels of irradiance to the receivers.

Robinson and Fitter (1999) argued that there was as yet no evidence of C transfer from AM fungi to the shoots of the receiver plant. They suggested that the low levels of ^{14}C detected in the shoots of receiver plants (e.g. Francis and Read 1984) originated from $^{14}CO_2$ respired by roots and microbes and re-fixed during photosynthesis by the receiver plant. In our experiment, labelling was performed at bud burst of the receivers and the chased period (7 days) ended after leaf expansion of sugar maple. Photosynthetic $^{14}CO_2$ fixation would not explain our findings as photosynthetic rates are restricted in developing leaves, as in *Acer campestre* (Küppers 1984). Furthermore, the quasi-absence of radioactivity in non-labelled

E. americanum, which would have had elevated photosynthetic rates during the chase period (Taylor and Pearcy 1976), argues against the possibility of net $^{14}CO_2$ fixation by photosynthesis in the receiver plants.

The close correlation between C allocated to the corm of *E. americanum* and C translocated to sugar maple saplings suggests that *E. americanum* exports C once its own carbohydrate storage is completed emphasising again the importance of source-sink relationship in C transfer. At the time of labelling 8 of the 12 *E. americanum* had apparently not completely filled their starch reserve, while 3 *E. americanum* plants were translocating small amounts of C to the corm, suggesting that the storage process was about to end in the former 8 *E. americanum*. We can estimate that if the labelling of *E. americanum* had occurred 2–3 days later, more *E. americanum* would have then completed their storage of carbohydrates and we would have observed higher ^{14}C movements towards sugar maple saplings. The magnitude of C transfer between *E. americanum* and sugar maple saplings is probably dependent on the climatic conditions prevailing at spring between the unfurling of *E. americanum* leaves and the canopy closure.

In spring, the *E. americanum* plants were highly mycorrhizal. The colonisation levels were, however, less than reported by Brundrett and Kendrick (1990b) and by Lapointe and Molard (1997) who found mycorrhizal levels of 67% and 82.8% respectively. Nevertheless, the presence of AM fungi in every tested plant reflected a sufficient inoculum potential in the experimental pots.

In autumn, the ^{14}C detected in 7 of the 22 *E. americanum* root systems strongly supports the notion that AM fungi living on sugar maple roots can be a source of mycorrhization of *E. americanum* roots. If the source of activity had been sugar maple root exudates then all *E. americanum* would have been equally labelled. Therefore, we concluded that about one-third of *E. americanum* plants were connected to the donor sugar maples in autumn 2001.

Notwithstanding, autumn labelling did not demonstrate whether C was transferred from sugar maple saplings to *E. americanum* root cells. Micro-autoradiographs with nuclear emulsion did not show any traces of radioactivity. The quantity of $^{14}CO_2$ we used for labelling donor plants (2×185 kBq per plant) was very low compared to the micro-autoradiography of Bücking and Heyser (2001) who used 7.4 MBq (200 μCi) $^{14}CO_2$ per plant. In our work, ^{14}C detection threshold levels were below background. Hence, radioactivity in fungal and root cells was not visually documented.

Possible transfer of C in autumn from sugar maple saplings to *E. americanum* for the root formation was less clear than the spring case of C transfer from *E. americanum* to maple saplings. However, traces of ^{14}C were detected in some of the *E. americanum* plants, suggesting a possible payback of C from sugar maples to *E. americanum* in autumn.

In conclusion, we have documented C transfer between sugar maple saplings and the spring ephemeral *E. americanum* connected by AM mycelium that appears reciprocal. An initial transfer occurs in spring from *E. americanum* to sugar maple saplings during leaf expansion of the saplings. A reciprocal transfer is highly likely in autumn. This second transfer was from the sugar maple saplings to newly developed roots of *E. americanum* plants, but we do not have conclusive proof at this point that imported C was used for the formation of *E. americanum* roots. Reciprocal transfers could be frequent in natural ecosystems where plants with different phenologies share AM fungi.

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