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Carbon and nitrogen flow in silver birch and Norway spruce connected by a common mycorrhizal mycelium

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Abstract Spruce and birch seedlings were grown together in boxes filled with unsterile peat. Both seedlings were colonized by the ectomycorrhizal fungus *Scleroderma citrinum.* The two plants thus shared a common external mycelium. ^{15}N-labelled ammonium was supplied exclusively to the fungus, while the birch or the spruce plant was continuously fed with ^{13}C-labelled CO_2 for 72 h. The carbon and nitrogen transfer rates were strikingly different for birch and spruce seedlings. The mycorrhizal mycelium received carbohydrates mainly from the birch plant and the nitrogen transfer by the fungus to the plants was largely directed towards the birch. Carbon assimilates were also transferred in both directions between birch and spruce; however, there was no conclusive evidence for a net transfer of carbon between the plants.

Key words Fungus · ^{15}N · ^{13}C · Mycelial links · *Scleroderma citrinum*

Introduction

In the ectomycorrhizal association, the fungus gains carbohydrates from the associated plant while much of the plant nutrient uptake is mediated through the fungus (Harley and Smith 1983). The inoculum potential of an already established mycelium is high. Thus, a growing seedling radicle in a forest has a high probability of becoming colonized by fungal mycelium already associated with another plant (Brownlee et al. 1983; Fleming 1984; Francis and Read 1984). Mycelial networks connecting two or more plants, belonging to the same or to different species, have been directly observed in transparent observation chambers. Further, it has been demonstrated that mycorrhizal links can provide pathways for, for example carbon and nitrogen transfer between plants (Brownlee et al. 1983; Finlay and Read 1986; Arnebrant et al. 1993). Read et al. (1985) suggested that transfer of nutrients from established plants to seedlings may be a crucial factor enabling young plants to survive in nutrient-limited situations. Newman (1988) summarized several possible benefits of a seedling becoming connected to an already established mycelial network, two of which are examined in the present study: (1) the seedling may obtain mineral nutrients from a mycorrhizal fungus whose organic carbon is supplied by another plant, and (2) the seedling may receive organic carbon via the mycorrhizal links, supplementing its own photosynthetic carbon gain.

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Materials and methods

Mycorrhizal synthesis

Ectomycorrhizal associations were synthesized between spruce *Picea abies* (L.) Karst. seedlings and the ectomycorrhizal fungus *Scleroderma citrinum* Pers. by the method described by Duddridge (1986) and modified by Finlay et al. (1988). The fungus was isolated from a sporocarp in a stand of *Fagus silvatica* L, Scania, Sweden. In the collection at the Department of Microbial Ecology, Lund University, this isolate referred to as No. 89.010. The mycorrhizal seedlings were transferred to polystyrene boxes, 230 × 230 × 18 mm deep (screening plates, Nunc A/S, Roskilde, Denmark), and incubated with the plants growing horizontally in a growth cabinet with 200 μmol m^{-2} s^{-1} PAR, 80% relative humidity and a 18 h/6 h and 18 °C/16 °C day/night cycle. Nonsterile, unfertilized and sieved (4-mm mesh) peat with a pH (H_2O) of 4.0 was used as growth substrate. The mycelium was allowed to extend from the roots and to colonize the peat substrate. After 2 months, a 1-month-old *Betula pendula* Roth seedling was planted in each box. The root systems of the two seedlings were allowed to mix freely and within 2 months the root systems of the birch seedlings were highly mycorrhizal. The boxes were left for another 6 months to allow the fungal mycelium to grow over a po-

lystyrene barrier into areas of peat from which plant roots were excluded.

Experimental conditions

The shoots of the two plants in a box were separately sealed in two glass chambers. Air containing 575 ppm CO_2 was fed to the chambers at a flow rate of 2 l h^{-1}. Labelled CO_2 was supplied to one chamber while the other chamber received unlabelled CO_2. Air containing ^{13}C-labelled CO_2 was generated by leading humid, CO_2-free air through a 250-ml bottle that contained 75 ml of a 1 M lactic acid solution. A 0.050 M $Na_2{}^{13}CO_3$ (99.1 atom % excess, Isotec Inc, Miamisburg, USA) solution was injected into the bottle at a flow rate of 1.0 ml h^{-1}. Air containing unlabelled CO_2 was generated correspondingly.

When the chambers were sealed, 1 mg of labelled nitrogen was added to the partitioned area of peat where only fungal mycelium was present. The labelled nitrogen was added as 67 μmol of $^{15}NH_4Cl$, (95 atom % excess, Merck, Sharp & Dome Ltd, Montreal, Canada), dissolved in 5 ml of water. An incubation period of 72 h was allowed for $^{13}CO_2$ and $^{15}NH_4^+$ assimilation and translocation. During this period $^{13}CO_2$ was continuously administered to one of the plants in a box, while the other plant received unlabelled CO_2.

At harvest, plants were partitioned into root and shoot and external mycelium were collected. No attempt was made to separate the plant root from fungal tissue in and on the root. Plant roots and shoots and fungal mycelium were lyophilized, weighed and ground in a ball mill prior to the ^{15}N and ^{13}C analyses. The total N and C and the isotopic labelling analyses were performed with an Automated Nitrogen Carbon Analyzer-Mass Spectrometer, (ANCA-MS, Europa Scientific Ltd) by Isotec Inc, Miamisburg, USA.

In 5 boxes the birch plant was fed with $^{13}CO_2$ while in another 5 boxes the spruce plant was fed with $^{13}CO_2$. However, plant roots growing under the barrier into the root-excluded compartment was observed at harvest in two of the boxes. These boxes were not included in the data analyses.

Table 1 Dry weights, proportion of biomass allocated to the shoot and nitrogen concentrations (dry wt.) of ectomycorrhizal seedlings of birch and spruce ($n=8$)

	Plant weight		Biomass allocated to the shoot (%)	N concentration	
	Shoot (mg)	Root (mg)		Shoot (%)	Root (%)
Birch	75	181	31	1.08	1.21
Spruce	120	106	52	0.88	0.81
$F_{[1,14]}$	3.4	4.3	22.6	1.1	1.8
ANOVA	$P=0.09$	$P=0.06$	$P<0.001$	$P=0.31$	$P=0.21$

Results and discussion

At harvest, root and shoot nitrogen concentration and total plant biomass were not significantly different for birch and spruce plants (Table 1). The proportion of plant biomass allocated to the shoot was, however, higher in spruce than in birch.

The birch seedling became colonized through contact with established *S. citrinum* mycelium. Hence initially, during the first 2 months of incubation, the spruce plant was the sole carbon source for the mycelium. However, 8 months later, during the ^{15}N and ^{13}C labelling period, the mycelium was almost entirely dependent on the birch plant for current photosynthates (Table 2). On average, 93% of the total amount of ^{13}C recovered in the external mycelium originated from the birch. Further, the transfer of ^{15}N by the ectomycorrhizal fungus to the plants was largely directed towards the birch (Table 3). On average, 94% of the total amount of ^{15}N recovered in the plants was found in the birch. Harley and Smith (1983, p. 397) argued that plants connected by a common mycorrhizal network would compete for nutrients derived from the fungal system and that most likely plant competitive ability would reside in the process of extracting most from and giving least to the fungus. However, in the present study, the plant that supplied the fungus with most organic carbon also obtained the most nitrogen from the fungus.

There is no obvious explanation of why the birch plant was the main carbon source for the fungus or also the main sink for its soil-derived nitrogen. Both the birch and the spruce plants were photosynthetically active and the root systems of both plants were heavily mycorrhizal. We did not estimate the growth rate of the two plants, and one explanation might be that the birch plants were in a more active growth state and thus showed a stronger demand for nitrogen. It is also possible that the rather low light intensity had some influence on carbon allocation in the system. A detailed study of the plant-fungus interface might have shed light on the observed differences in carbon and nitrogen flows.

The total amount of carbon transferred from the birch to the spruce (root + shoot) was 4.2 μmol C. This

Table 2 The carbon flow in a birch and a spruce seedling linked together by a common mycorrhizal network of *Scleroderma citrinum*. The birch or the spruce plant was fed labelled CO_2 (donor plant). The amount of ^{13}C recovered in the donor and receiver plants and in the mycelium was analyzed (spruce donor plant $n=5$, birch donor plant $n=3$)

Plant fed with $^{13}CO_2$	Amount of ^{13}C in donor plant		Amount of ^{13}C in receiver plant		Amount of ^{13}C in mycelium
	Shoot (μmol ^{13}C)	Root (μmol ^{13}C)	Shoot (μmol ^{13}C)	Root (μmol ^{13}C)	(μmol ^{13}C)
Birch	95.2	68.4	2.0	2.2	6.3
Spruce	74.2	27.7	0	1.5	0.5
$F_{[1,6]}$	0.47	2.0	7.27	0.08	7.4
ANOVA	$P=0.52$	$P=0.21$	$P<0.05$	$P=0.78$	$P<0.05$

Table 3 The amount of ^{15}N recovered in ectomycorrhizal seedlings of birch and spruce. Labelled ammonium was supplied to a root-excluded area of peat colonized by mycorrhizal mycelium ($n=8$)

	Amount of ^{15}N recovered in a plant	
	Shoot (μmol ^{15}N)	Root (μmol ^{15}N)
Birch	0.79	2.78
Spruce	0.07	0.13
$F_{[1,14]}$	10.6	14.4
ANOVA	$P=0.01$	$P=0.01$

corresponds to 2.2% of birch net carbon assimilation. Nearly 50% of the carbon transferred to the spruce was recovered in the shoot (Table 2). The carbon flow in the reverse direction, from the spruce to the birch, was 1.5 μmol and not significantly lower ($F_{[1,6]}$, $P=0.34$). Thus the carbon flow was bidirectional and there is no firm evidence for a net carbon transfer. However, no labelled carbon was recovered in the birch shoot when the spruce plant was fed $^{13}CO_2$ (Table 2). This might indicate that a substantial amount of labelled carbon was retained in fungal tissue in/on the birch root.

Read and coworkers have studied the transport of ^{14}C-labelled assimilates between *Pinus* sp. plants connected by a common ectomycorrhizal mycelium (Brownlee et al. 1983; Read et al. 1985; Finlay and Read 1986). Autoradiography revealed that when $^{14}CO_2$ was fed to the shoot of a donor plant, labelled assimilates were distributed from the donor plant to the external mycelium and further into the mycorrhizal roots of the receiver plant. A quantitative analysis showed that 0.2–1.0% of donor plant net carbon assimilation was transferred, within 3–5 days, to the mycorrhizal receiver plant. Most of the labelled carbon transferred was recovered in the root of the receiver plant with only minor amounts in the shoot. The amount of labelled carbon recovered in nonmycorrhizal receiver plants was 10–100 times lower than the amount recovered in mycorrhizal receiver plants. It was also shown that significantly higher amounts of radioactivity were transferred to the roots of shaded receiver plants than to the roots of plants in full light. These experiments did not unequivocally demonstrate the occurrence of a net flow of carbon assimilates to the receiver plant. Recently, in a dual-labelling field experiment, it was reported that a substantial net flow of carbon occurred from paper birch to Douglas fir via mycorrhizal mycelium (Simard et al. 1995). In this study, three different shading levels were applied to Douglas fir seedlings and it was observed that the level of irradiance strongly influenced the carbon transfer between the plants.

However, of the possible benefits for a plant to become connected an already established mycelium examined in the present study, none was confirmed, i.e. the plant that supplied the fungus with most organic carbon did also obtain most nitrogen from the fungus, and there were no conclusive evidence for a net transfer of carbon between the plants.

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