

the soil core. Root, detrital and soil carbon content were determined by combustion/gas chromatography (Europa Scientific); combustion/gas chromatographic determinations of shoot carbon content values from the peak biomass harvest in 1992 were used to calculate shoot carbon pools. Microbial carbon content was determined by chloroform fumigation.

Microcosm experiment. Seeds of plants representative of the serpentine and sandstone grassland communities were planted in 0.4-m diameter tubes with a 0.95-m deep column of serpentine soil in September–October 1992 and grown in open-top enclosures supplied with ambient (360 p.p.m.) or elevated (720 p.p.m.) CO₂. By the second growing season, all were similar in plant species composition and ecosystem properties, and so are combined for presentation and analysis. Nitrogen, phosphorus and potassium (20 g m⁻²) were supplied once each season as a slow-release fertilizer to half of the tubes in a factorial design. In mid-March 1993, a subset of the tubes was pulse labelled by closing the chambers and supplying 1 litre of 99% ¹³C₂ to the chamber atmosphere during a 5-h period (8:00 to 13:00). At the height of flowering the following year (18–20 April 1994), we determined the δ¹³C of soil CO₂ (¹³CO₂ BR) collected from perforated stainless steel tubes inserted into the soil (0–15 cm depth) and the amount and δ¹³C of CO₂ produced by soil heterotrophs during 96-h laboratory incubations of root-free soil (¹³CO₂ HR). We assumed that root δ¹³C was the same as the δ¹³C of CO₂ produced by root respiration (¹³CO₂ RR) and calculated the proportional contributions from roots (y) and soil heterotrophs (1 - y) to below-ground respiration: ¹³CO₂ BR = y(¹³CO₂ RR) + (1 - y)(¹³CO₂ HR). We also calculated root respiration by difference, as below-ground respiration rates²² minus CO₂ production rates in the soil incubations. The ¹³C signal associated with the CO₂ enrichment of the atmosphere and the pulse label allowed us further to partition the heterotrophic respiration flux into three components. The soil organic carbon (SOC) contribution to heterotrophic respiration (HR) was calculated using the elevated CO₂ treatment with no pulse label, where x is the proportional contribution of SOC to HR: ¹³CO₂ HR = x(¹³C SOC) + (1 - x)(¹³C plant). Rhizodeposition and previous year's litter contributions to HR were calculated using ambient and elevated CO₂ treatments that had been pulse labelled, where y is the proportional contribution of rhizodeposition to HR and x is from above: ¹³CO₂ HR = x(¹³C SOC) + y(¹³C rhizodeposition) + (1 - x - y) (¹³C litter).

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Net transfer of carbon between ectomycorrhizal tree species in the field

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Different plant species can be compatible with the same species of mycorrhizal fungi^{1,2} and be connected to one another by a common mycelium^{3,4}. Transfer of carbon^{3–5}, nitrogen^{6,7} and phosphorus^{8,9} through interconnecting mycelia has been measured frequently in laboratory experiments, but it is not known whether transfer is bidirectional, whether there is a net gain by one plant over its connected partner, or whether transfer affects plant performance in the field^{10,11}. Laboratory studies using isotope tracers show that the magnitude of one-way transfer can be influenced by shading of 'receiver' plants^{3,5}, fertilization of 'donor' plants with phosphorus¹², or use of nitrogen-fixing donor plants and non-nitrogen-fixing receiver plants^{13,14}, indicating that movement may be governed by source–sink relationships. Here we use reciprocal isotope labelling in the field to demonstrate bidirectional carbon transfer between the ectomycorrhizal tree species *Betula papyrifera* and *Pseudotsuga menziesii*, resulting in net carbon gain by *P. menziesii*. *Thuja plicata* seedlings lacking ectomycorrhizae absorb small amounts of isotope, suggesting that carbon transfer between *B. papyrifera* and *P. menziesii* is primarily through the direct hyphal pathway. Net gain by *P. menziesii* seedlings represents on average 6% of carbon isotope uptake through photosynthesis. The magnitude of net transfer is influenced

by shading of *P. menziesii*, indicating that source–sink relationships regulate such carbon transfer under field conditions.

Plants within communities can be interconnected and exchange resources through a common hyphal network^{1–14}, and form guilds based on their shared mycorrhizal associates^{15–17}. Consequently, the theory that plant community dynamics operate mainly within the constraints of resource supply¹⁸ should be reformulated to consider mutualism between plants and their mycorrhizal fungi, as well as microbially mediated resource sharing^{15,17,19}.

We have studied shared compatibility of ectomycorrhizal fungi and interspecific carbon transfer in *Betula papyrifera* Marsh. and *Pseudotsuga menziesii* (Mirb.) Franco. Seven ectomycorrhizal morphotypes were common between *B. papyrifera* and *P. menziesii*, covering over 90% of their root tips, indicating a high potential for interspecific hyphal connections²⁰. We used reciprocal labelling with ¹³C and ¹⁴C in the field to examine below-ground transfer between the two tree species. To our knowledge, this is the first study in which reciprocal labelling has been used to investigate bidirectional and net carbon transfer.

We examined carbon transfer during two growing seasons, when seedlings planted in forest soil were two or three years old. Ectomycorrhizal *B. papyrifera* and *P. menziesii* and vesicular–arbuscular (VA) mycorrhizal *Thuja plicata* D. Don. were planted 0.5 m apart in three-seedling groups. These three tree species occur naturally together in the experimental area. *Thuja plicata* served as a marker for indirect isotope movement through soil pathways (that is, in respired gaseous CO₂, exudates, and sloughed root and fungal cells). Four or six weeks before labelling, *P. menziesii* seedlings in the replicate groups were exposed to three light treatments: deep shade, partial shade, and full ambient sunlight (Table 1). After the shading period, *B. papyrifera* and *P. menziesii* in each group were pulse-labelled with gaseous ¹³CO₂ or ¹⁴CO₂. This approach enabled detection of the carbon isotope that was received by one seedling from the other. Reciprocal labelling schemes were applied to replicate seedling groups in the first year to account for differences in absolute isotope fixation between ¹³C and ¹⁴C. Pulse-labelling for 2 h in full ambient light with a minimum photosynthetically active radiation level of 800 μmol m⁻² s⁻¹ was followed by a 9-day chase period under the light treatments. Seedlings were then harvested and separated into foliage, stems, coarse roots and fine roots. Ground tissue was combusted to CO₂ and analysed for ¹³C abundance by mass spectrometry and ¹⁴C by liquid scintillation.

Carbon transfer between *B. papyrifera* and *P. menziesii* occurred in both directions in the first and second year, and, although there was no net transfer in the first year, there was a net gain in carbon by *P. menziesii* in all three light intensities in the second year (Table 1 and Fig. 1). In the first year (two-year-old seedlings), isotope transfer to *P. menziesii* was balanced by reciprocal transfer to *B. papyrifera* (so there was no net transfer) in all light intensities ($P = 0.10$ for light treatment effects). Bidirectional transfer between *B. papyrifera* and *P. menziesii* represented 4% of total isotope fixed by both species together, and transfer through soil pathways to *T. plicata* was <1% of the total transfer between the ectomycorrhizal species²⁰.

In the second year (three-year-old seedlings), net transfer to *P. menziesii* and bidirectional transfer between *B. papyrifera* and *P. menziesii* represented on average 6% and 7%, respectively, of the total isotope fixed by the two tree species together, and both were over twice as great in deep shade as in partial shade or full light ($P < 0.05$; Table 1 and Fig. 1). Transfer to VA mycorrhizal *T. plicata* averaged 18% of the total transfer between *B. papyrifera* and *P. menziesii*, suggesting that most transfer between the ectomycorrhizal tree species occurred through hyphal connections²⁰. The greater effect of shading on magnitude of bidirectional transfer and the occurrence of net transfer in the second rather than the first experiment year coincided with greater root extension and potential for interplant hyphal linkages, as well as increased vigour of

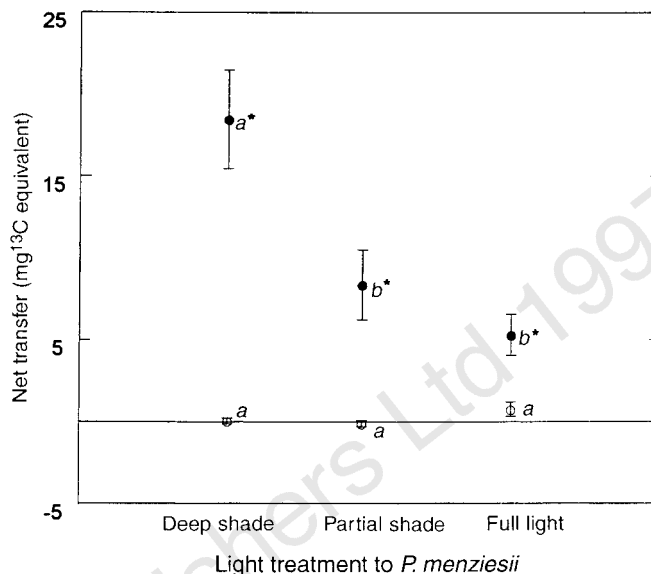


Figure 1 Mean (\pm s.e.) net transfer from *B. papyrifera* to *P. menziesii* in deep shade, partial shade and full ambient sunlight in the first (two-year-old seedlings, open circles) and second (three-year-old seedlings, filled circles) experiment years. Within years, means denoted by the same letter (a or b) do not differ significantly ($P < 0.05$). Asterisks indicate treatments in which net transfer was greater than zero ($P < 0.05$).

seedlings in the second year, and indicates that transfer varies according to seedling status and environmental conditions.

The greater transfer in deep shade in the second year was due to increased transfer from *B. papyrifera* to *P. menziesii*, indicating that net transfer was affected by changes in the sink strength of *P. menziesii*. Net transfer from *B. papyrifera* to *P. menziesii* was associated with whole-seedling net photosynthetic rates 1.5 and 4.3 times greater for *B. papyrifera* than for *P. menziesii* in full light and deep shade, respectively, and foliar nitrogen concentrations twice as great for *B. papyrifera* as for *P. menziesii*²⁰. These results suggest that carbon was transferred between species down carbon and nitrogen gradients. Fully developed leaves are strong sinks for nitrogen and are sources rather than sinks for carbon²¹, and amino acids rather than sugars have been shown to pass from ectomycorrhizal fungi into host plants^{22,23}. Consequently, it has been suggested that transfer may be regulated more strongly by a nitrogen gradient than a carbon gradient^{6,22}.

The 3–10% net transfer from *B. papyrifera* to *P. menziesii* measured in these experiments represents a substantial carbon gain by *P. menziesii*. It is similar to the 10% ¹⁴C transfer one way from clonal parent plants to connected ramets that has been suggested to be of sufficient magnitude to increase survival and growth of the ramets²⁴. It is also similar to the 5–15% transfer of fixed gaseous ¹⁵N₂ from *Alnus glutinosa* to *Pinus contorta* through ectomycorrhizal connections⁵, and to the 0–10% of carbon in *Cynodon dactylon* roots that was derived from *Plantago lanceolata* through VA mycorrhizal connections²⁵. Carbon transfer of this magnitude, especially if it is associated with nitrogen, would be expected to affect performance under critically harsh conditions or over the lifetime of a *P. menziesii* seedling^{10,11}.

In our experiments, the average amount of received isotope that was retranslocated from roots to foliage was 13% for *P. menziesii* and 45% for *B. papyrifera*. The amounts retranslocated from roots to foliage of receivers exceed those observed in previous ectomycorrhizal carbon-transfer experiments (usually <10%)^{5,10}, and could either supplement photosynthate or, if carbon is transferred as amino acids, the foliar nitrogen pools²³. The pattern of carbon

Table 1 Light treatment, photosynthetic rate and carbon transfer

Light treatments to <i>P. menziesii</i>	PAR incident on <i>P. menziesii</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)*	Whole-seedling net photosynthetic rate of <i>P. menziesii</i> ($\mu\text{mol s}^{-1}$)	Bidirectional carbon transfer ($\text{mg }^{13}\text{C}$ equivalent)	Bidirectional transfer as % of total isotope in donor <i>B. papyrifera</i> + <i>P. menziesii</i>	Net carbon transfer as % of total isotope in donor <i>B. papyrifera</i> + <i>P. menziesii</i>
Two-year-old seedlings					
deep shade	15 ± 5 c	0.012 ± 0.002 b	0.97 ± 0.29 a	2.7	0.1
partial shade	75 ± 5 b	0.021 ± 0.005 a	1.19 ± 0.20 a	2.6	-0.3
full ambient light	175 ± 7 a	0.023 ± 0.023 a	2.13 ± 0.59 a	6.9	2.4
Three-year-old seedlings					
deep shade	60 ± 10 c	0.05 ± 0.01 b	22.06 ± 2.88 a	10.7	9.5
partial shade	250 ± 60 b	0.18 ± 0.04 a	10.87 ± 2.04 b	5.3	4.3
full ambient light	1,330 ± 50 a	0.14 ± 0.03 a	7.98 ± 1.65 b	3.9	2.7

Photosynthetically active radiation (PAR) and whole-seedling net photosynthetic rate of *P. menziesii* under three light treatments, and calculated bidirectional carbon transfer, and per cent bidirectional and net carbon transfer between *B. papyrifera* and *P. menziesii*. Values with different letters indicate significant differences among light treatments at $P < 0.05$; d.f. = 7 for two-year-old seedlings, and d.f. = 4 for three-year-old seedlings. Values are mean ± s.e.

* PAR and net photosynthetic rate were measured on a uniformly cloudy day in the first year (two-year-old seedlings) and a sunny day in the second year (three-year-old seedlings).

transfer in these ectomycorrhizal plants therefore contrasts with that reported for some VA mycorrhizal systems, where relatively little of the transferred carbon was onwardly mobilized into shoots of receiver plants, and may represent carbon restricted to fungal tissue in roots of receiver plants^{3,25,26}.

The amount of carbon exchanged between *B. papyrifera* and *P. menziesii* is indicative of a tightly linked plant–fungus–soil system. Our study extends earlier laboratory results^{3–6,10,11,24,25} to the field, providing direct evidence for both bidirectional and net carbon transfer between plant species, for the occurrence of hyphal as well as soil pathways, and for source–sink regulation of net transfer in field conditions. When *P. menziesii* seedlings are establishing and growing in the shade of illuminated *B. papyrifera* in natural, mixed communities, they may benefit directly from their association with *B. papyrifera* through supplemental gains in transferred carbon. There is a further possibility that carbon is distributed below-ground among plants across resource gradients, other than light, that affect relative photosynthetic potential within a mycorrhizally linked plant community. Such a mechanism would offer one explanation for the ability of species-rich communities to maintain productivity during drought or where nutrients are limiting²⁷. If our results reflect the magnitude of carbon transfer in natural systems, then the net competitive effect of one species on another cannot be predicted without a better understanding of interplant carbon transfer through shared mycorrhizal fungi and soil pathways. A more even distribution of carbon among plants as a result of below-ground transfer may have implications for local interspecific interactions²⁸, maintenance of biodiversity^{11,15,17}, and therefore for ecosystem productivity, stability and sustainability^{16,19,27}. □

Methods

Seedling groups. In both experiment years, each experimental unit consisted of a seedling group. In the first year (two-year-old seedlings), a 3 × 2 factorial set of treatments was replicated four times in a completely randomized design (24 experimental units). The treatments consisted of three *P. menziesii* light treatments (full ambient light, partial shade and deep shade) and two labelling schemes (¹⁴C-*B. papyrifera* with ¹³C-*P. menziesii*; ¹³C-*B. papyrifera* with ¹⁴C-*P. menziesii*). In the second year (three-year-old seedlings), each of the three light treatments was applied to five replicate groups of seedlings in a completely randomized design (15 experimental units). Owing to a shortage of replicate seedling groups, only one labelling scheme was applied in the second year: ¹⁴C-*B. papyrifera* with ¹³C-*P. menziesii*.

Light treatments. In the partial and deep shade treatments, *P. menziesii* seedlings were shaded with lightly and heavily clothed cone-shaped tents four weeks before labelling on 14–16 July 1993 (first year), and six weeks before labelling on 4–5 August 1994 (second year). The shade tents were removed from *P. menziesii* during the 2-h pulse for maximum labelling efficiency. Each *B. papyrifera* and *P. menziesii* seedling was sealed inside flexible, airtight, 5-mm thick × 60-cm wide × 90-cm tall fluoropolymer gas sampling bags (Norton Performance Plastics). The shoot of one partner seedling was then pulsed with 200 ml gaseous ¹³CO₂ (99%, equivalent to 107.42 mg ¹³C), and the shoot of the

other partner was pulsed with 7.4 MBq gaseous ¹⁴CO₂ (equivalent to 52.86 μg ¹⁴C, released from Na₂¹⁴CO₃ with lactic acid).

Bidirectional and net transfer calculations. Sample δ¹³C (‰) and ¹⁴C (Bq) values were converted to excess carbon isotope (mg) for bidirectional and net transfer calculations. The conversions were based on previously described procedures^{29,30}. Conversion of δ¹³C (‰) to the absolute isotope ratio (¹³C/¹²C) of the sample was based on the PeeDee Belemnite (PDB) standard. Fractional abundance (¹³C/¹³C + ¹²C) and total carbon content (mg) of the sample were used to calculate ¹³C content (mg) of the sample. Conversion of ¹⁴C (Bq) to ¹⁴C content (mg) was based on the batch-specific activity (λ) of Na₂¹⁴CO₂ (λ = 1.96 GBq mmol⁻¹; Amersham Canada). Excess isotope (¹³C or ¹⁴C) content (mg) of the tissue was calculated as the product of excess isotope content of the sample and tissue biomass, and excess isotope (mg) of the whole plant was determined by summing excess isotope content of the four tissue types. Bidirectional and net transfer calculations were based on whole-plant levels of excess isotope that were received from partner donor seedlings. Bidirectional transfer was the sum of isotope received by both *P. menziesii* and *B. papyrifera* in a seedling group. Conversely, net transfer was the difference between isotope received by *P. menziesii* and that received by *B. papyrifera*. Positive net transfer indicated that a greater amount of isotope was received by *P. menziesii* than by *B. papyrifera*, and negative net transfer indicated the opposite. Bidirectional and net transfer were expressed as proportions of total isotope assimilated by *P. menziesii* and *B. papyrifera* together.

Statistical analysis. Significant labelling scheme effects in the first year were detected using two factor analysis of variance (ANOVA) (α set at 0.05). For calculations of bidirectional and net carbon transfer, labelling scheme effects were first removed by applying correction factors to excess ¹⁴C content (mg) on a treatment–species–tissue basis. The correction factors were the species–tissue-specific ratios of excess ¹³C (mg) to excess ¹⁴C (mg) measured in the reciprocal labelling schemes of the same light treatment. The treatment–species–tissue-specific correction factors were averaged over the four replicates per labelling scheme in the first year. The correction factors derived in the first year were applied to data collected in the second year, because only one labelling scheme was applied in the second year. Using the corrected excess ¹⁴C values (analogous to excess ¹³C equivalent values), data from the first and second years were subject to one-factor ANOVA for comparisons of net and bidirectional transfer among *P. menziesii* light treatments (7 degrees of freedom for the first year, 4 for the second). Net transfer estimates were compared with zero using *t*-tests.

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Dissociating prefrontal and hippocampal function in episodic memory encoding

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Human lesion data indicate that an intact left hippocampal formation is necessary for auditory–verbal memory¹. By contrast, functional neuroimaging has highlighted the role of the left prefrontal cortex^{2–4} but has generally failed to reveal the predicted left hippocampal activation. Here we describe an experiment involving learning category–exemplar word pairs (such as ‘dog...boxer’) in which we manipulate the novelty of either individual elements or the entire category–exemplar pairing.

We demonstrate both left medial temporal (including hippocampal) and left prefrontal activation and show that these activations are dissociable with respect to encoding demands. Left prefrontal activation is maximal with a change in category–exemplar pairings, whereas medial temporal activation is sensitive to the overall degree of novelty. Thus, left prefrontal cortex is sensitive to processes required to establish meaningful connections between a category and its exemplar, a process maximized when a previously formed connection is changed. Conversely, the left medial temporal activation reflects processes that register the overall novelty of the presented material. Our results provide striking evidence of functionally dissociable roles for the prefrontal cortex and hippocampal formation during learning of auditory–verbal material.

The role of medial temporal structures, including the hippocampal and parahippocampal formation, in episodic memory is well established, with homologous left- and right-sided structures mediating verbal and visual aspects of memory, respectively^{1,5,6}. In the monkey, lesions restricted to the hippocampus lead to striking memory impairment⁷. Memory processes can be fractionated into those relating to encoding (learning), consolidation (storage) and retrieval (recall). Functional neuroimaging, although failing to specify the role of the medial temporal cortex, has emphasized specific involvement of the left and right prefrontal cortices at encoding and retrieval respectively^{2–4}. In this experiment we sought to reconcile prefrontal and hippocampal involvement in encoding by showing that hippocampal activation reflects a response to the relative novelty of sensory data whereas prefrontal activation reflects an aspect of associative semantic processing. By novelty we refer to the relative familiarity of the verbal material in the context of the experiment; associative semantic processing refers to the formation of meaningful associations between verbal items.

The study material consisted of word-paired associates comprising a category (for example, dog) and an exemplar (for example, boxer) presented auditorily. Twelve separate perfusion scans were acquired in six male subjects while they were presented with sets of 12 paired associates. The scanning window was preceded by the presentation of two sets of 12 paired associates that created the context for the critical experimental manipulation during scanning (Fig. 1). We used a design in which category and exemplar were independent factors, with two levels related to whether an item was novel (that is, not previously presented) or old (previously presented). The study thus comprised four separate conditions representing two levels of novelty (new versus old) with respect to either category or exemplar (Fig. 1). So, for example, the initial presentations of a word pair (such as dog ... boxer) during the lead-in period might be followed, during the critical scanning period, with an entirely new pair (new–new condition: for example, food ... biscuit). Alternatively, it may be followed by a change in category (new–old condition, such as sportsman ... boxer) or a change in exemplar (old–new condition: for example, dog ... labrador). In the fourth condition, subjects heard repeat presentation of pairs presented during the lead in (old–old condition; that is, dog ... boxer). Thus, in the new–new condition, the relative emphasis is on novelty, whereas in the new–old and old–new conditions, the process emphasized is the making and breaking of semantic linkages.

Activation of the left dorsolateral prefrontal cortex (DLPFC) was sensitive to a manipulation of the association between category and exemplar or vice versa. Thus, maximal activation in this region was seen in the two conditions involving a change in category exemplar pairings. Figure 2a shows a statistical parametric map (SPM) of this activation for the contrast of the two conditions (new–old and old–new) in which there was a change in category–exemplar linkage with the condition where there was no change (old–old) ($P < 0.05$, corrected). These data are shown in Fig. 2b, where the mean adjusted activity in the left DLPFC is plotted for each of the four

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