In contrast to the presumably partially aquatic, *Proganochelys*[5], *Australochelys* was found in a deposit with terrestrial vertebrates that is thought to represent an arid environment[1], suggesting that by the Early Jurassic turtles were ecologically diverse. □

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Mycorrhizae alter quality and quantity of carbon allocated below ground

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PLANTS and soils are a critically important element in the global carbon–energy equation. It is estimated that in forest ecosystems over two-thirds of the carbon is contained in soils and peat deposits[1]. Despite the importance of forest soils in the global carbon cycle, fluxes of carbon associated with fundamental processes and soil functional groups are inadequately quantified, limiting our understanding of carbon movement and sequestration in soils. We report here the direct measurement of carbon in and through all major pools of a mycorrhizal (fungus–root) coniferous seedling (a complete carbon budget). The mycorrhizal symbiont reduces overall retention of carbon in the plant–fungus symbiosis by increasing carbon in roots and below-ground respiration and reducing its retention and release above ground. Below ground, mycorrhizal plants shifted allocation of carbon to pools that are rapidly turned over, primarily to fine roots and fungal hyphae, and host root and fungal respiration. Mycorrhizae alter the size of below-ground carbon pools, the quality and, therefore, the retention time of carbon below ground. Our data indicate that if elevated atmospheric CO_2 and altered climate stressors alter mycorrhizal colonization in forests, the role of forests in sequestering carbon could be altered.

Ectomycorrhizae are one form of fungus–root symbioses whose morphological characteristics include a root enclosed in a sheath of fungal tissue and a complex network of hyphae between root epidermal and cortical cells that extends into the soil[2,3]. The intimate morphology extends the surface area for carbon and nutrient exchange between symbionts, and increases the area in contact with soil compared with the non-mycorrhizal condition. Until now, complete carbon budgets for any mycorrhizal symbiosis were unobtainable because of the lack of suitable measuring techniques[4]. Previous carbon allocation models were based on differences between entire non-inoculated and mycorrhizal seedlings, and not on calculations using direct measures of fungal processes and respective fungal mass. Estimates of carbon retention and respiration by hyphae of the vesicular arbuscular mycorrhizal (VAM) symbiosis range from 4% to 20% (refs 5–8). Ectomycorrhizal hyphal respiration was estimated as 30% of below-ground respiration[9]. However, specific rates were not calculated because hyphal biomass was not quantified.

TABLE 1 Fraction dry weights, and relative activity of fractions adjusted to 10^5 Bq total ^{14}C assimilation by needles

Fraction	Non-inoculated	Ectomyc.	Non-inoculated	Ectomyc.
	Dry weight (mg)		Relative activity (Bq ^{14}C mg^{-1})	
Total plant	2,080	1,878*		
Above ground				
				Respiration
Total	940	868*	50.0	44.0
				Remaining
Bud and stem	266	238	20.0	22.4
Needles	674	630	27.0	27.0
Below ground				
				Respiration
Total	1,137	1,010	15.3	22.1**
Roots (host)	1,137	910***	15.3	19.8
Active hyphae†	n.p.	100***	n.p.	40.1***
				Remaining
Coarse roots	936	741**	14.6	17.7
Fine roots (host)	201	169	16.9	23.4*
Active hyphae	n.p.	100***	n.p.	32.8***

Values are means of numbers that were calculated using individual seedling data of ^{14}C allocation to fractions and the respective fraction dry weight. The individual seedling relative activity value was then standardized to 10^5 Bq total radioisotope assimilation by needles. All statistical conventions are as in Fig. 2. n.p., Not present.
† See Fig. 2 legend for estimates of hyphal mass in ectomycorrhizal root tips.

We studied the ectomycorrhizal association between ponderosa pine (*Pinus ponderosa* Laws.) seedlings and *Hebeloma crustuliniforme* (Bull.: St. Amans) Qúel that developed in root–mycocosms (Fig. 1, and refs 10, 11). Root–mycocosms allowed us to measure carbon fluxes through a portion of intact hyphae while symbiotic integrity was maintained. Photosynthesis of shoots and respiration of all structural fractions were first measured, and seedlings were then used to determine allocation and respiration of recently fixed ^{14}C (ref. 10). Fluxes from host and fungus were calculated after direct measurements were made on the fungus and various host fractions. A complete carbon budget was then constructed using respective fraction mass.

When all ^{14}C retention values were summed, mycorrhizal seedlings retained 39.8% of the ^{14}C fixed by needles and non-inoculated seedlings retained 41.3% (Fig. 2). The small amount of active fungal mass (5% of total mycorrhizal seedling dry weight) increased ^{14}C allocation below ground by about 23% compared with non-inoculated seedlings. The fungus received about 7% of the total ^{14}C fixed. As the fungus contributed a small amount to total seedling dry weight, our data suggest that the fungus did not receive an inordinate amount of carbon (high carbon cost to the host has been a widespread speculation). Sixty per cent of the ^{14}C allocated to the fungus, or 4.3% of total ^{14}C assimilated, was respired by the fungus. Hyphal respiration represented 19.4% of total below-ground respiration. The hypothesis that a small amount of fungus has a substantial effect on carbon allocation within mycorrhizal plants[7,12,13,14] is supported by our data.

We calculated that bacterial respiration was less than 2% of hyphal respiration or 0.2% of total below-ground respiration, and ^{14}C release as root exudation was negligible (Fig. 2). Therefore, these fluxes were considered insignificant in our budget and are included with the respective pool.

Total above-ground ^{14}C respiration by mycorrhizal seedlings was about 10% less than non-inoculated seedlings, whereas below-ground respiration of mycorrhizal seedlings was about 35% greater. Above-ground ^{14}C allocation values between

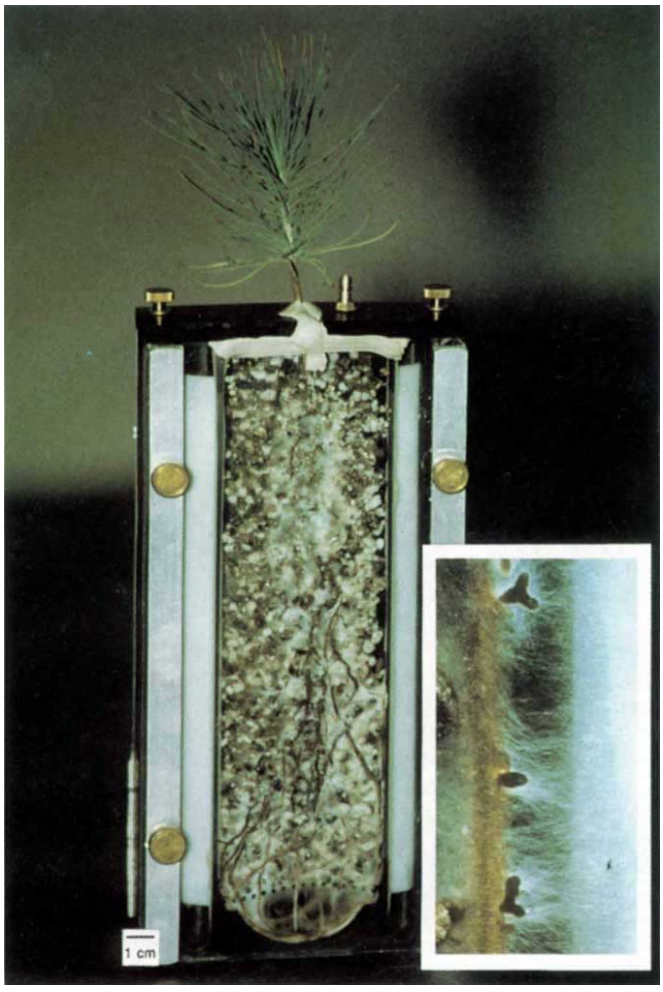

FIG. 1 Ponderosa pine (*Pinus ponderosa* Laws.) seedling (6–7-month-old) in symbiosis with *Hebeloma crustuliniforme* in a root–mycocosm. Insert shows extramatrical hyphae emanating from ectomycorrhizae and passing from the root compartment to one of the fungal compartments.

METHODS. Isolate S-260 of *H. crustuliniforme* (USDA Forest Sciences Laboratory, Corvallis, Oregon, USA; obtained from Mycorr Tech Inc., Pittsburgh, Pennsylvania, USA) and ponderosa pine seedlings were grown in root-mycocosms (2.5 cm thick, 11.5 cm wide and 23 cm long) in the growth chamber as previously described[10,11]. Hyphae passed from the root compartment into the fungal compartments through the gap created between the glass face plate and the wall separating the two types of compartments. Hyphae in the fungal compartment grew onto glass-fibre filter paper backed by Nitran membrane and rockwool. Seedlings were prepared for ^{14}C pulse-chase and gas exchange experiments by removing the glass face plate and applying hydrated lanolin to the surfaces of the black plastic block that are in contact with the glass (including over the hyphae passing between compartments) so that each compartment was hermetically sealed allowing the gas exchange of each symbiont to be independently measured. A completely randomized design was used to assign eight seedlings to two treatments, with and without fungus. The experiments were replicated once in time ($n=8$) and all dependent data were subjected to analysis of variance (ANOVA) to test for fungal treatment effects[19]. Residual analyses indicated that the usual normality and homogeneity of variance assumptions were satisfied[20].

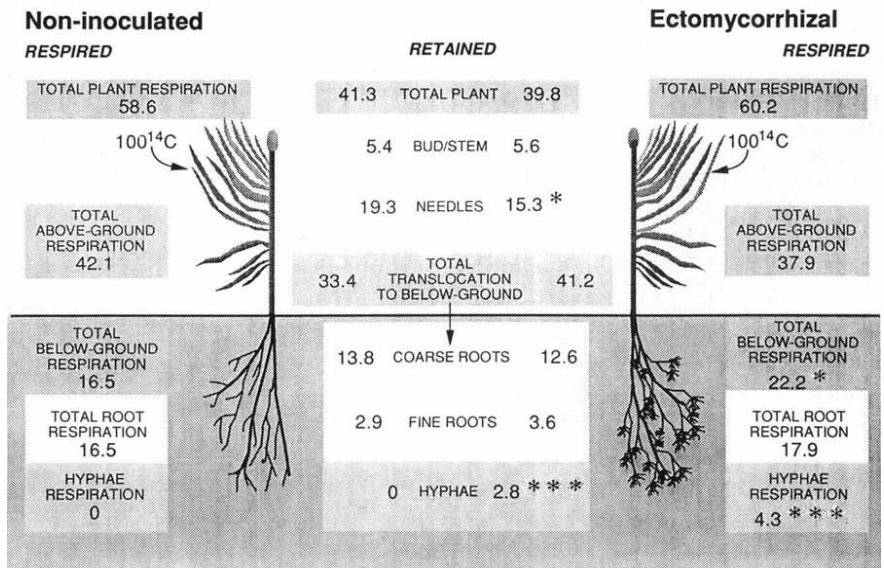

FIG. 2 Flow of ^{14}C (Bq ^{14}C allocated during the chase period per 100 Bq ^{14}C assimilated by needles) through seedlings. Differences between fungal treatments significant at: $*\alpha = 0.15$, $**\alpha = 0.10$, $***\alpha = 0.05$.

METHODS. Seedlings were labelled for 1 h with 1,850 kBq NaH $^{14}CO_3$, respiration gases were collected during a 72 h translocation period, seedlings harvested, and tissue and respiration samples analysed for radioisotope[10,14]. Below-ground plant parts were classified as fine roots ($\leqslant 1$ mm), mycorrhizae, or coarse roots. Total and active hyphal mass in root compartments of experimental seedlings, and active hyphae in the fungal compartments of a matched set of root–mycocosms, were estimated using light microscopy and fluorescein diacetate (FDA)[21]. Roughly 6% and 100% (by weight) of hyphae in the root and fungal compartments took up FDA, respectively. Total hyphal mass in the fungal compartments was determined gravimetrically using the difference in mass of the filter paper before installation and at harvest. Hyphal mass of mycorrhizal seedlings was calculated using the active mass in root and fungal compartments, and of the Hartig net and sheath of the mycorrhizae (30% of the dry weight of an ectomycorrhiza, ref. 3, p. 128). Specific rates in the pulse-chase and gas exchange experiments were based on direct measures for fungus-only and fungus-plus-host fractions of inoculated seedlings, and for the host-only fractions of non-inoculated seedlings. Host root rates in symbiosis were inferred. No active bacteria were found (FDA staining[21]) in the fungal compartments and an average of 2 mg were found in the root compartment. We assumed similar hyphal and bacterial respiration rates to calculate the bacterial contribution to below-ground respiration. Below-ground compartments were washed with 0.1M KCl to collect extractable ^{14}C-labelled exudates[14]. Values were calculated by adjusting total ^{14}C assimilation for each seedling type to 100 Bq, then the proportional adjustment was applied to each fraction.

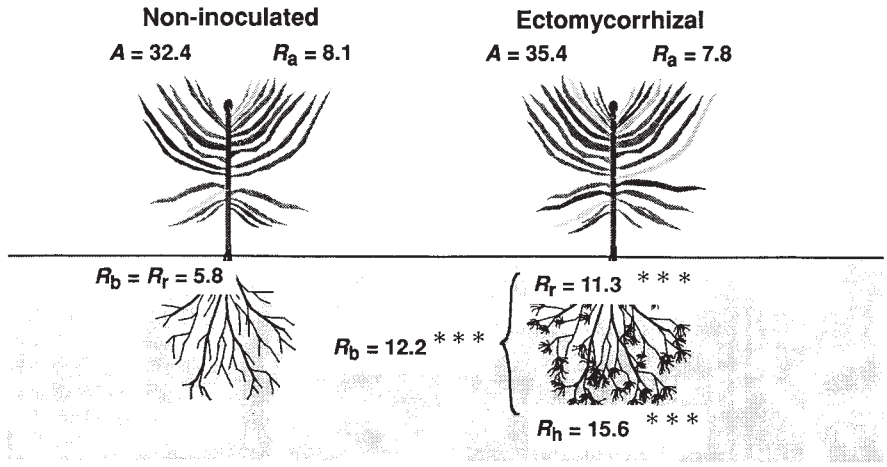

FIG. 3 Rates of respiration and net assimilation of CO_2 in non-inoculated *Pinus ponderosa* seedlings and seedlings colonized with *Hebeloma crustuliniforme*. Statistical conventions as in Fig. 2. Units are: Above-ground, *A*, assimilation, nmol CO_2 per g dry weight top per s; R_a, respiration, nmol CO_2 per g dry weight top per s; Below-ground, R_r, respiration of roots, nmol CO_2 per g dry weight per s; R_h, respiration of hyphae, nmol CO_2 per g dry weight active hyphae per s; R_b, total below-ground respiration, nmol CO_2 per g dry weight roots and hyphae per s.
METHODS. Assimilation and respiration were measured using a closed-loop infrared gas analyser (IRGA) 24±1 °C (ref. 10). Above-ground respiration was measured after steady-state conditions were achieved in the dark using a shade cloth. Below-ground respiration was measured after removing the cuvette from the IRGA and connecting the inflow and outflow lines directly to hose barbs of the below-ground compartments to form a closed loop. Each below-ground compartment was first scrubbed with CO_2-free air to achieve a standard CO_2 concentration similiar to ambient of the laboratory. This condition was maintained for a minimum of 16 to 20 volume changes of respective compartments before commencing respiration measurements.

seedling types were similar. However, there was a trend (P = 0.15) towards lesser retention by needles in the mycorrhizal seedlings, as noticed previously[14].

Because allocation is also a function of fraction size, we standardized the allocation values by dry weight to make direct comparisons of relative fraction activity (Table 1). In general, the relative activity of the below-ground fraction of mycorrhizal seedlings was greater than non-inoculated seedlings. Specifically, the highest relative activities below ground occurred in pools of mycorrhizal seedlings that are rapidly turned over and returned to the atmosphere, for example hyphae and fine roots. Fungal hyphae had the greatest relative activity of all below-ground fractions.

Hyphae had the greatest respiration rates of any seedling fraction; *H. crustuliniforme* respired 15.6 nmol CO_2 per g dry weight active hyphae per second (Fig. 3). These are the first reported rates of respiration of ectomycorrhizal hyphae in symbiosis using fungal mass that was directly quantified.

The total below-ground respiration rate of mycorrhizal seedlings was 2.1 times the rate of non-inoculated seedlings (Fig. 3). The greater rate is due to at least three factors. First, the hyphae had the highest respiration rate, as noted above. Second, colonized roots had inherently higher calculated respiration rates compared with non-inoculated roots, indicating a 'colonization respiration' for ectomycorrhizal host roots. Colonization respiration has been estimated only for VAM-colonized roots[15] and is demonstrated for tissue infected by pathogens ('infection respiration')[16]. Third, mycorrhizal seedlings had a greater percentage of active fine roots (fine root/coarse root weight ratio: 1.32 versus 1.16 in mycorrhizal and non-inoculated seedlings, respectively). Collectively, the three processes contribute to the more rapid release of photosynthetically fixed carbon in mycorrhizal plants.

There was an indication that mycorrhizal seedlings accumulated less biomass than non-inoculated seedlings (P = 0.15; Table 1), resulting from slightly less retention in seedling tops, a shift in allocation from coarse roots to fine roots in mycorrhizal seedlings, and the production of hyphae. Conifers in symbiosis with *H. crustuliniforme* accumulate less mass despite similar photosynthetic rates compared with non-inoculated seedlings which may be related to the carbon allocated to form an extensive hyphal network outside the root[13]. Our data indicate that to account fully for the differences in mass between seedling types, the respiration of the fungus and the colonization respiration of host roots must be considered. These fluxes illustrate the importance of symbionts in below ground and total plant carbon dynamics, even when the symbiont mass is relatively small.

Small changes in carbon use in plants can result in large shifts in carbon cycling at larger scales. Changes in the colonization of roots by mycorrhizal fungi, or in the proportional allocation of carbon to form coarse and fine roots may affect the sequestration of carbon in ecosystems. Factors such as elevated CO_2 also increase carbon incorporation into below-ground pools with short residence times such as fine roots[17,18]. Regardless of how elevated CO_2 influences mycorrhizal colonization and activity, the contribution of mycorrhizal systems to the total carbon budget of forests must be evaluated if accurate estimates of carbon fluxes at larger scales are to be made. For our data to be of most use in models, adjustments may be needed for soil temperature, precipitation, seasonal root and mycorrhizae formation and growth, and the diversity of plant species/fungal species associations. Investigations are underway to characterize partitioning of carbon below ground under various future climates to improve model predictions of carbon sequestration[1]. □

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