An ecosystem-scale radiocarbon tracer to test use of litter carbon by ectomycorrhizal fungi

Kathleen K. Treseder a,*, Margaret S. Torn b, Caroline A. Masiello c

a Department of Ecology and Evolutionary Biology and Department of Earth System Science, University of California, Irvine, CA 92697, USA
b Earth Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA
c Department of Earth Science MS 126, Rice University, 6100 Main St, Houston, TX 77005, USA

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Abstract

The degree to which ectomycorrhizal fungi rely on decomposing litter as a carbon source in natural ecosystems is unknown. We used a radiocarbon (14C) tracer to test for uptake of litter carbon by ectomycorrhizal fungi as part of the Enriched Background Isotope Study (EBIS) in Oak Ridge Reservation, Tennessee. In EBIS, leaf litter from a highly 14C-labeled Quercus alba (white oak) forest was reciprocally transplanted with litter from a nearby low-labeled forest that had not been as strongly exposed to 14C. These litter transplants were conducted yearly. We measured 14C signatures of ectomycorrhizal fungi collected from each forest four months and 2.25 years after the first litter transplant. The ectomycorrhizas were associated with white oak trees. We found no significant differences in 14C signatures of ectomycorrhizal fungi exposed to low-labeled versus high-labeled litter, indicating that less than 2% of the carbon in ectomycorrhizal biomass originated from transplanted litter. In contrast, ectomycorrhizal 14C signatures from the high-labeled forest were 117–140‰ higher than those from the low-labeled forest. This pattern suggests that ectomycorrhizal fungi acquired most (or all) of their carbon from their host plants, probably via direct transfer of photosynthate through the roots.

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1. Introduction

Ectomycorrhizal fungi possess most characteristics typical of decomposer organisms. For instance, this group can grow aseptically on media containing only organic forms of nitrogen or phosphorus (Melin and Norkrans, 1948; Abuzinadah and Read, 1986; 1989b). They can access these organic nutrients by releasing into the soil such extracellular enzymes as proteases, polyphenol oxidases, cellulases, phosphatases, and lignin peroxidases (Lundeberg, 1970; Giltrap, 1982; Dighton, 1991; Read, 1991; Cairney, 1999), although they are less effective at metabolizing more recalcitrant compounds than are other fungi (Read, 1991; Colpaert and van Tichelen, 1996; Leake et al., 2002; Read and Perez-Moreno, 2003). In addition, ectomycorrhizal fungi are physiologically capable of absorbing amino acids and small peptides from the soil solution, owing to the presence of specific transporter proteins in the plasma membrane (Chalot and Brun, 1998).

Typically, host plants provide carbohydrates to their ectomycorrhizal symbionts in exchange for nutrients (Smith and Read, 1997). Nevertheless, ectomycorrhizal fungi also possess the capability to acquire carbon directly from litter as they assimilate organic nutrients. Isotope tracer studies have demonstrated uptake of carbon from soil by ectomycorrhizal fungi under greenhouse conditions (Abuzinadah and Read, 1989a; Finlay et al., 1996; Taylor et al., 2004). These findings have led to suggestions that ectomycorrhizal fungi may use soil or litter carbon for biomass construction, or as an energy source (e.g. Chalot et al., 1994; Chalot and Brun, 1998). Direct field-based tests of symbiotic carbon use by ectomycorrhizal fungi are scarce, however. As a result, it is not clear whether this process is prevalent under natural conditions.

We took advantage of a unique isotope labeling experiment to examine the extent to which ectomycorrhizal fungi use litter carbon in an intact ecosystem, in order to better understand environmental controls over ectomycorrhizal growth and activity. In this experiment, we compared radiocarbon signatures of ectomycorrhizal fungi grown in the presence of
labeled litter (that had been collected fall 2000) was evenly excluded from fall 2000 onward. Instead, high-labeled or low-treatment in each site. In each plot, natural litterfall was in the high-labeled site. There were four 7 m plots for each site (Hanson et al., 2005). Briefly, treatments be termed ‘high-label’ and ‘low-label’ litter, respectively. Likewise, litter collected from these sites will referred to as the ‘high-label’ site, and Walker Branch as the ‘low-label’ site. Likewise, litter collected from these sites will be termed ‘high-label’ and ‘low-label’ litter, respectively.

A reciprocal litter transplant was conducted between the two sites, as described in Hanson et al. (2005). Briefly, treatments were established in a factorial design, with high-labeled litter in the high-labeled site, low-labeled litter in the low-labeled site, high-labeled litter in the low-labeled site, and low-labeled litter in the high-labeled site. There were four 7 × 7 m plots for each treatment in each site. In each plot, natural litterfall was excluded from fall 2000 onward. Instead, high-labeled or low-labeled litter (that had been collected fall 2000) was evenly spread on each plot at a rate of 500 g dry mass m⁻² y⁻¹ in May 2001, February 2002, and February 2003. Nine months after the first litter additions, Hanson et al. (2005) observed that ¹⁴C signatures of the Oi horizon (i.e. recognizable litter) diverged significantly between litter treatments for both sites. Specifically, ¹⁴C values were 219 ± 11 ‰ SE for the low-labeled litter/low-labeled site treatment; 660 ± 19 ‰, high-labeled litter/low-labeled site; 308 ± 20 ‰, low-labeled litter/high-labeled site; and 802 ± 79 ‰, high-labeled litter/high-labeled site (Hanson et al., 2005). If ectomycorrhizal fungi were using litter-derived carbon to construct new biomass, we expect that the ¹⁴C signatures of the ectomycorrhizal root tips should differ between litter treatments.

2.2. Sample collection

We isolated ectomycorrhizal root tips from soil cores collected in September 2001 and August 2003. An ectomycorrhizal root tip is produced when an ectomycorrhizal fungus encompasses a newly-forming root with fungal tissue. The resulting structure contains approximately 40% fungal tissue and 60% plant tissue (Allen, 1991). These ectomycorrhizal root tips are the site of nutrient and carbon transfer between the fungus and plant (Smith and Read, 1997). We focused on the ectomycorrhizal root tips because they are the most recognizable structure of ectomycorrhizal fungi in these soils. White oak is colonized by ectomycorrhizal fungi; red maple is arbuscular mycorrhizal. We did not identify the species of ectomycorrhizal fungi present, though Cenococcum graniforme grows on white oak in Oak Ridge (O’Neill et al., 1987), and Pisolithus tinctorius is a common associate of white oak (Walker and McLaughlin, 1991). For each sampling time, we collected 9.5 cm diameter by 5 cm deep cores from two random locations in each plot. Cores were compiled within each plot, for 16 samples total for each date.

From each sample, we extracted roots by sieving soils through a 1 mm mesh screen. Roots were washed three times with deionized water and examined under an Olympus SZX4 stereoscope (Olympus Microscopes, Melville, NY) at 30× magnification. We used fine forceps to pluck ~2 mg of ectomycorrhizal root tips from each sample. In some cases, we could not isolate enough ectomycorrhizal tissue to perform the radiocarbon analyses, so not every plot was included in each sampling time. Specifically, six plots were omitted in 2001, and one plot in 2003. The isolated tips were sonicated for 5 min in a solution of 3.95% sodium metaphosphate (w/v), rinsed three times with deionized water, and lyophilized in a Labconco Freezone 4.5 freeze-drier (Labconco, Kansas City, Missouri) for 24 h.

2.3. Radiocarbon analyses

Accelerator mass spectrometry (AMS) was used to conduct radiocarbon measurements of ectomycorrhizal root tips. Analyses were performed at the Center for AMS, Lawrence Livermore National Laboratory. Samples were combusted to CO₂ with cupric oxide and silver in quartz tubing at CAMS or the Torn Lab. The resulting CO₂ was recovered cryogenically over an iron catalyst (Vogel et al., 1987). ¹⁴C signatures were calculated relative to a universal standard (oxalic acid I, decay-corrected to 1950) in permil (‰) (Stuiver and Polach, 1977; Donahue et al., 1990). Results were corrected to a δ¹³C value of −25‰, so that mass dependent isotope fractionation did not affect results reported as Δ¹⁴C. Higher Δ¹⁴C signatures indicate larger ¹⁴C/¹²C ratios. Our samples were analyzed with a precision of ±7.0‰, based on instrument error.

2.4. Statistics

To test our hypothesis that ectomycorrhizal fungi form a significant fraction of their biomass from litter carbon, we conducted a separate fully-factorial ANOVA for each sampling date. The dependent variable was Δ¹⁴C signature of ectomycorrhizal root tips. Independent factors were site (low-labeled versus high-labeled) and litter type (low-labeled versus high-labeled). Kolmogorov–Smirnov and Fmax tests indicated that
our data met the assumptions of ANOVA for both sampling times (Sokal and Rohlf, 1995), so we performed statistical analyses on untransformed data. Differences between groups were considered significant when \( P < 0.05 \). Significant differences in \( \Delta^{14}C \) values between treatments would support our hypothesis.

3. Results

Contrary to our hypothesis, we found no evidence that ectomycorrhizal fungi acquired carbon from decomposing litter. Specifically, \( \Delta^{14}C \) values of ectomycorrhizal root tips were not higher in plots with high-labeled litter (Fig. 1; 2001: \( F_{1,1} = 2.619, P = 0.157 \); 2003: \( F_{1,1} = 1.136, P = 0.309 \)). Instead, plant hosts (via roots) appeared to be the sole source of carbon incorporated into ectomycorrhizal root tips. Ectomycorrhizal \( \Delta^{14}C \) signatures from the high-labeled site were 117–140‰ higher than those from the low-labeled site, depending on the date (Fig. 1; 2001: \( F_{1,1} = 84.593, P < 0.001 \); 2003: \( F_{1,1} = 51.568, P < 0.001 \)).

This pattern may reflect signatures of soluble carbon transferred directly to ectomycorrhizal fungi from host plants. Namely, soluble carbon of host plants was \( ^{14}C \)-enriched in the high-labeled site, and we were able to track this label to the ectomycorrhizal root tips. Evidence for enrichment of soluble plant carbon in the high-labeled site includes the enrichment of leaves (to 100%\(^{14}C\)) produced in the first spring following the \( ^{14}C \) release (Hanson et al., 2005), and \( \Delta^{14}C \) values of root respiration that were consistently 100%\(^{14}C\) higher than those of the low-labeled site throughout 2002–2003 (Cisneros-Doval et al., in press). Both leaves and root respiration should have been produced from soluble carbon in plants. The enriched soluble carbon may have been partially composed of stored carbon originally fixed in summer 1999, and then recycled within the plant.

The signal size and analytical precision within our study allowed us to detect as little as 2% transplanted litter carbon in ectomycorrhizal biomass. To calculate this detection limit, we used a two-member mixing model: \( \%C_{\text{litter}} = (\Delta^{14}C_{\text{ECM}} - \Delta^{14}C_{\text{other}})/(\Delta^{14}C_{\text{litter}} - \Delta^{14}C_{\text{other}}) \). \( \Delta^{14}C_{\text{ECM}} \) is the signature of ectomycorrhizal fungi that had been exposed to transplanted litter; \( \Delta^{14}C_{\text{other}} \), the signature of all other carbon sources; \( \Delta^{14}C_{\text{litter}} \), the signature of transplanted litter; and \( \%C_{\text{litter}} \), the percentage carbon derived from transplanted litter. For the high-labeled litter/low-labeled site treatment, \( \Delta^{14}C_{\text{litter}} \) was 1005‰. Other potential carbon sources for ectomycorrhizal fungi could have included photosynthate directly transferred from the host plants, pre-existing litter, and soil. These sources likely encompassed a range of \( \Delta^{14}C \) values, but we could assume that the \( \Delta^{14}C \) of ectomycorrhizal fungi from the low-labeled litter/low-labeled site treatment (2001 average: 178%\(^{14}C\); 2003 average: 94%\(^{14}C\)) would represent an integration of all these sources. We used this value for \( \Delta^{14}C_{\text{other}} \). Because our sampling precision was 7‰ (instrument error), we could detect use of transplanted litter carbon if \( \Delta^{14}C_{\text{ECM}} - \Delta^{14}C_{\text{other}} \) was at least 14‰. By solving the mixing model, we found that \( \%C_{\text{litter}} \) equaled 2% in the low-labeled site for both sampling dates; this value represented our detection limit.

4. Discussion

The relatively large differences in \( \Delta^{14}C \) signatures between transplanted litter and other carbon sources in the EBIS project, compared to instrument error, enabled us to detect contributions of carbon from transplanted litter to ectomycorrhizal biomass that were as small as 2%. Because additions of differently-labeled litter did not significantly alter the \( ^{14}C \) signatures of ectomycorrhizal root tips, ectomycorrhizal fungi in the Oak Ridge Reservation did not appear to use the transplanted litter as a significant source of carbon. Instead, they seemed to rely on the transfer of carbon from host plants for most—if not all—of the carbon used for construction of fungal biomass.

If ectomycorrhizal fungi had incorporated carbon from the transplanted litter, we would expect this activity to be especially apparent in the August 2003 sampling. At this point, the first cohort of added litter would have been decomposing for 2.25 years; the second, for 1.5 years; and the third, for 0.5 years. Based on a decomposition model derived from empirical tests of litter decomposition rate in the Oak Ridge Reservation (Hanson et al., 2005),
approximately 710 g dry biomass m\(^{-2}\) should have been lost from the 1500 g m\(^{-2}\) transplanted litter by August 2003. Likewise, \(\Delta^{14}C\) values of the Oi horizon in January 2002 indicated that 45% of litter mass had turned over within the first nine months after litter transplants in the EBIS experiment (Hanson et al., 2005). Cisneros-Dozal et al. (in press) observed a consistent increase in \(^{14}C\) signatures of soil respiration in the high-labeled litter plots compared to those of low-labeled litter plots for both sites. They calculated that during the period of May 2002 through March 2004, between 1 to 42% (depending on moisture levels) of total soil respiration was derived from decomposition of the transplanted litter. Evidently, litter-derived carbon was available for metabolism by soil microbes, yet the ectomycorrhizal fungi did not exploit this carbon source.

The 2003 results bear further consideration, because the ectomycorrhizal fungi had been exposed to the transplanted litter for two winters at the time of sampling. All trees in the Oak Ridge Reservation are deciduous, so translocation of carbon to roots is sharply reduced during the winter. According to minirhizotron data, only about 15% of annual fine root growth in the Reservation occurs during winter and in early spring, before leaf out (Joslin et al., 2001). Thus, carbohydrate supply to ectomycorrhizal fungi from host plants was likely to be reduced during the two winters. Ectomycorrhizal fungi may have slowed down their metabolic rate, decreased their growth rate, and/or accessed stored carbon during the winter, instead of using soil carbon.

Although we did not detect uptake of litter carbon by ectomycorrhizal fungi, previous greenhouse-based studies have documented this phenomenon. Abuzinadah and Read (1989a) estimated that 9% of carbon in Betula pendula seedlings could be derived from organic compounds in the growth media, but only in the presence of the ectomycorrhizal fungus Hebeloma crustuliniforme. In addition, Finlay et al. (1996) recorded uptake and incorporation of alanine-C into Betula roots colonized by Paxillus involutus. These studies demonstrate that at least two species of ectomycorrhizal fungi have the potential to access soil carbon in association with Betula seedlings. Nevertheless, this potential was not realized in our field setting. The discrepancy between our study and those of Abuzinadah and Read (1989a) and Finlay et al. (1996) could result from differences in the scale of the experiment (greenhouse versus field), the plant species involved (birch versus white oaks), the stages of plant growth (seedling versus adult), or the ectomycorrhizal species involved (P. involutus and H. crustuliniforme versus a community of ectomycorrhizal fungi).

Others have reported indirect evidence for soil carbon use by ectomycorrhizal fungi in intact ecosystems, based on natural abundance \(^{14}C\) signatures of ectomycorrhizal fungi or host plants. Hobbie et al. (2002) collected two samples of Douglas fir needles in an Oregon forest in 1998. These needles displayed \(\Delta^{14}C\) values of 104±4‰ (precision) and 109±4‰ (Hobbie et al., 2002), which are enriched compared to atmospheric \(\Delta^{14}C\) values of 97±2–5‰ over Schauinsland, Germany in that year (Levin et al., 2003). In addition, they reported that \(^{14}C\) signatures of ectomycorrhizal mushrooms collected from the Oregon forest in 1997 were 110±4‰, also more enriched than the 1997 atmosphere over Schauinsland (100±2–5‰). The investigators suggested that the \(^{14}C\) enrichment of needles and mushrooms could be attributed to direct uptake of relatively \(^{14}C\)-enriched soil carbon via ectomycorrhizal fungi, and they estimated that up to 7% of needle carbon could have been obtained via this mechanism. Likewise, Chapela et al. (2001) proposed that soil carbon contributed to ectomycorrhizal biomass in a radiata pine plantation in Ecuador, based on a \(^{14}C\) signature of 98‰±4‰ (precision) for ectomycorrhizal fruiting bodies collected in 1997. The investigators mentioned that this signature was comparable to that of the atmosphere in 1996 (data source not given), implying that some portion of the ectomycorrhizal carbon was at least 1 year old. They suggested that soil was a source of this older carbon. Nevertheless, the ectomycorrhizal signature is also similar to the Schauinsland atmospheric signature of 100±2–5‰ in 1997, the same year the fruiting bodies were collected. Thus, another possible interpretation of the ectomycorrhizal \(\Delta^{14}C\) signatures is that the fungi were primarily accessing carbon that had been recently fixed by their host plants. It can be difficult to use natural abundance measurements of radiocarbon to quantify soil carbon uptake by ectomycorrhizal fungi, because the difference between soil and plant carbon sources may be of similar magnitude to sampling error. In addition, atmospheric \(^{14}CO_2\) values can differ among regions of the globe by as much as 10‰ (Randerson et al., 2002). As a result, attempts to determine the age of tissue carbon by comparing \(^{14}C\) signatures from Schauinsland with those of tissues from Oregon or Ecuador may be subject to additional uncertainty. The studies of Hobbie et al. (2002) and Chapela et al. (2001) were also unable to rule out the possibility that photosynthate was stored in the host tree for a year or more before allocation to roots and ectomycorrhizal fungi.

Organic nitrogen uptake by ectomycorrhizal fungi may be inhibited by high availabilities of inorganic nitrogen (Nordin et al., 2001), which could result in minimal litter carbon use. Nevertheless, this mechanism may not be operating at Oak Ridge Reservation; potential nitrogen mineralization rates are relatively low across most of that landscape, particularly in the forests (Garten and Ashwood, 2002). In addition, nitrogen fertilization stimulates tree growth in sycamore plantations in the reservation (Luxmoore et al., 1993). Plant growth appears to be nitrogen limited in our study site.

Even though the ectomycorrhizal root tips from our study did not contain significant amounts of litter-derived carbon, these results do not necessarily preclude temporary uptake of organic nutrients by the fungi. For example, it is possible that the ectomycorrhizal fungi absorbed radiocarbon-labeled amino acids or peptides, but that the carbon contained within the organic nutrients was respired during or shortly after uptake. In general, ectomycorrhizal roots display higher respiration rates per gram tissue than do non-mycorrhizal roots (reviewed in Harley and Smith, 1983). In a greenhouse experiment with
14C-labeled alanine, Finlay et al. (1996) found that 60% of absorbed alanine-C was actively respired (instead of being incorporated into biomass) in root systems of birch colonized by P. involutus, compared with 28% in non-mycorrhizal birch roots. If the same ratio of 100:40 (incorporated:retained in biomass) applied to our field site, than our detection sensitivity of carbon fluxes from litter to ectomycorrhizal fungi would have been 5%. In other words, less than 5% of carbon taken up by ectomycorrhizal fungi would have been derived from transplanted litter.

Another possibility is that ectomycorrhizal fungi acquired carbon from litter, but allocated this carbon preferentially to extramatrical hyphae instead of root tips. Owing to technical constraints, we did not measure 14C signatures of extramatrical hyphae. Nevertheless, we are aware of no evidence to suggest that ectomycorrhizal fungi allocate carbon exclusively to hyphae versus root tips under any circumstances.

Our findings support the prevailing paradigm that ectomycorrhizal fungi rely predominantly on their host plants for carbon. This exclusivity has implications for the regulation of ectomycorrhizal activity. Specifically, ectomycorrhizal activity might vary primarily as a function of the amount of photosynthate allocated to ectomycorrhizal fungi by host plants, as well as nutrient requirements of the fungi. In contrast, the availability of labile carbon in the soil may not strongly influence ectomycorrhizal activity. Since ectomycorrhizal fungi release extracellular enzymes that break down organic compounds (Lundeberg, 1970; Giltrap, 1982; Dighton, 1991; Read, 1991; Cairney, 1999; Leake et al., 2002), they contribute to decomposition even if they do not use litter carbon. The decomposer activity associated with ectomycorrhizal fungi could be closely tied to plant allocation patterns, if the rate of production of extracellular enzymes is influenced by carbon flux through roots to fungi. In this respect, environmental controls over ectomycorrhizal activity may differ from those of other decomposer groups that rely on soil organic matter and litter carbon for energy. It remains to be seen whether predictions of decomposition rates can be improved by modeling ectomycorrhizal- and saprotrophic-mediated mineralization separately.

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