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

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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 (^{14}C) 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 ^{14}C-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 ^{14}C. These litter transplants were conducted yearly. We measured $\Delta^{14}C$ 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 ^{14}C 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 $\Delta^{14}C$ 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.

Keywords: Carbon; Decomposition; Ectomycorrhizal fungi; Host plant; Litter; Photosynthate; Radiocarbon; Roots; Saprotrophic

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 asymbiotic 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

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^{14}C-enriched host trees versus ^{14}C-enriched leaf litter. We hypothesized that a detectable portion of ectomycorrhizal carbon would be derived from plant litter, and thus from asymbiotic, or saprotrophic, carbon use.

2. Methods

2.1. Experimental design

We conducted this work as part of the Enriched Background Isotope Study (EBIS) based in Oak Ridge Reservation, Tennessee (35° 58′ N, 84° 16′ W). EBIS was established to take advantage of a local, large-scale release of radiocarbon from industrial incinerators in summer 1999 (Hanson et al., 2005). Nearby trees became radiocarbon-enriched after incorporating $^{14}CO_2$ into their tissues. In fall of 2000, fresh mixed litter dominated by *Q. alba* (white oak) and *Acer rubrum* (red maple) was collected from two locations: 'Tennessee Valley Authority (TVA) Chestnut Ridge' and 'Walker Branch'. Both sites are located on Ultisols. TVA Chestnut Ridge was closer to the release point, and produced litter with a Δ^{14}C of 1005 ± 19‰ SE (Cisneros-Dozal et al., in press; Hanson et al., 2005). Walker Branch was farther from the release point, so litter from this site was less enriched (221 ± 2‰ SE). Hereafter, TVA Chestnut Ridge will be 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 ^{14}C signatures of the Oi horizon (i.e. recognizable litter) diverged significantly between litter treatments for both sites. Specifically, Δ^{14}C 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 Δ^{14}C 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 tinctorus* 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 SZ40 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 CO_2 with cupric oxide and silver in quartz tubing at CAMS or the Torn Lab. The resulting CO_2 was recovered cryogenically and reduced to graphite through reaction with hydrogen gas over an iron catalyst (Vogel et al., 1987). Δ^{14}C 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 δ^{13}C value of −25‰, so that mass dependent isotope fractionation did not affect results reported as Δ^{14}C. Higher Δ^{14}C signatures indicate larger $^{14}C/^{12}C$ 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 Δ^{14}C 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 F_{max} tests indicated that