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LETTER

Ectomycorrhizal colonization slows root decomposition: the *post-mortem* fungal legacy

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Abstract

The amount of carbon plants allocate to mycorrhizal symbionts exceeds that emitted by human activity annually. Senescent ectomycorrhizal roots represent a large input of carbon into soils, but their fate remains unknown. Here, we present the surprising result that, despite much higher nitrogen concentrations, roots colonized by ectomycorrhizal (EM) fungi lost only one-third as much carbon as non-mycorrhizal roots after 2 years of decomposition in a piñon pine (*Pinus edulis*) woodland. Experimentally excluding live mycorrhizal hyphae from litter, we found that live mycorrhizal hyphae may alter nitrogen dynamics, but the afterlife (litter-mediated) effects of EM fungi outweigh the influences of live fungi on root decomposition. Our findings indicate that a shift in plant allocation to mycorrhizal fungi could promote carbon accumulation in soil by this pathway. Furthermore, EM litters could directly contribute to the process of stable soil organic matter formation, a mechanism that has eluded soil scientists.

Keywords

Decomposition, ectomycorrhizal roots, fine roots, immobilization, ^{15}N, *Pinus edulis*, soil organic matter.

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INTRODUCTION

If mycorrhizal symbionts consume an average of 15% of photosynthate globally, then plants allocate roughly 9 petagrams (9×10^{15} g) of carbon (C) to mycorrhizal fungi annually, which exceeds the sum of all human-caused C emissions. This vast amount of C, along with the recognition that mycorrhizal abundance responds to global change (Treseder 2004), has bolstered interest in the influence of mycorrhizal colonization on C cycling. Yet, the technical difficulty of measuring fungal turnover in soil has impeded reaching a consensus. Most dominant trees in temperate forests, including piñon pine (*Pinus edulis*), form ectomycorrhizal (EM) associations (Read *et al.* 2004). When EM fungi colonize plant roots, they form a sheath around the outside and penetrate among cortical cells so that EM roots are composed of roughly 20–40% fungal tissue (Hobbie 2006). Thus, temperate trees deposit great amounts of EM root litter when roots die. This EM litter input holds great importance in nutrient cycles, in addition to the C cycle, because EM roots tend to have higher nutrient concentrations than other roots (Langley & Hungate 2003). Although external hyphae appear stable in short-term laboratory incubations (Bååth *et al.* 2004; Wallander *et al.* 2004), the decomposition rate of EM root litter has never been measured under natural conditions.

Ectomycorrhizal fungi have long been known to influence decomposition either by competing with saprotrophic decomposers for soil resources (Gadgil & Gadgil 1971) or by producing enzymes that degrade complex organic matter (Read 1991). Evidence from pure-culture incubations suggests that live mycorrhizal fungi may play an important role in the decomposition of their own dead tissues (Kerley & Read 1998). In some ecosystems, particularly those with poor soils, mycorrhizal fungi can dominate microbial biomass and activity. If mycorrhizal fungi do indeed have primary access to their own litter, this closed nutrient cycle could represent a mechanism by which mycorrhizae sway the balance of competition between plants and free-living microbes (Langley & Hungate 2003).

In the present study, we compared decomposition rates of EM and non-mycorrhizal (NM) root litter in a piñon pine woodland. To isolate the influence of live EM hyphae on root litter decomposition, we buried litterbags in paired cores that were covered with fine-poresize mesh that allowed fungal ingrowth but excluded roots. One core of

each pair was rotated frequently to experimentally exclude in-growing hyphae. We compared the influence of mycorrhizal status of root litter (EM and NM) and the presence of live EM hyphae on C and N dynamics over 2 years of decomposition under field conditions.

METHODS

In August 2002, we harvested root litter from piñon pines at Sunset Crater National Monument 10 km NE of Flagstaff, AZ, where EM fungi commonly colonize 30–50% of root tips (Gehring & Whitham 1991) and EM root mass composes roughly one-fourth of total fine root mass. Roots from other species that occur in the soil below piñon canopies, predominantly *Fallugia paradoxa* or *Juniperus monosperma*, were removed based on visible differences in colour, thickness and architecture. We sorted fine piñon roots (< 1 mm diameter) according to EM status macroscopically and verified the distinction under a dissecting microscope. Litter was dried at 60 °C. Previous laboratory incubations of pinyon roots that had been surface sterilized and autoclaved (described in Langley & Hungate 2003) indicate that EM fungi on roots removed from the field and subjected to drying do not remain metabolically active. We placed 100 mg of EM root litter and 100 mg of NM root litter into separate nylon mesh litter bags. All litter bags were placed, along with displaced soil, in hyphal ingrowth cores similar to those described by Johnson *et al.* (2002).

Hyphal ingrowth cores

PVC pipe (5 cm diameter) was cut into cylinders 15 cm tall. Eight square windows totalling 70% of the surface area of each cylinder were cut from of each core. All cores were covered with 45-μm poresize mesh to allow the passage of hyphae but not roots (Fig. 1a). In November 2002, cores were implanted in the soil under the east and west sides of 16 piñon pine canopies at the dripline in pairs (Fig. 1b) within 1 km of the trees from which root litter was collected. One core from each pair remained static to allow hyphal ingrowth (**+H**), and the other was rotated periodically to sever ingrowing EM hyphae (**−H**). The −H cores, being exactly the same as the +H cores in construction, experienced the same bulk water movement. The mesh effectively restricted root growth in both types of cores. The −H cores were rotated at least once weekly during the warm and wet times in the monsoonal window (June to October) and at least once monthly during cold and dry months (November to May). Researchers using hyphal exclusion cores in clayey soils have expressed concerns that core rotation leaves air gaps in the soil surrounding the −H cores causing treatment artefacts (Johnson *et al.* 2002). The cinder soil at Sunset Crater has a large particle size and very low cohesion; it conformed rapidly to the edges of the cores following rotation and minimized artificial air gaps.

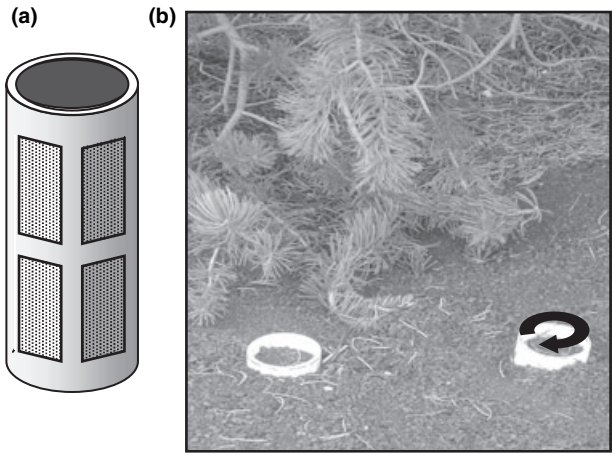

Figure 1 (a) Schematic of a hyphal ingrowth core, and (b) a photograph of core implementation in the field.

Sampling and analysis

At 6, 10 and 24 months after implantation, 6–10 pairs of cores were removed. We weighed 10 g subsamples of field-wet soil from each core, dried them at 105 °C for 72 h, and weighed them again to measure gravimetric soil moisture. Litter was removed from bags, dried at 60 °C, weighed, ground with a mortar and pestle, and analysed on a Carlo Erba elemental analyzer (Milan, Italy) coupled to a Finnegan Delta Plus mass spectrometer (Bremen, Germany) for C and N mass and isotopic composition. Samples of initial litter were analysed for C, N, lignin and phosphorus as well as scanned for specific root length (n = 3–5). Phosphorus was measured using a modified micro-Kjeldahl digestion (Parkinson & Allen 1975) followed by colorimetric analysis on a Lachat flow-injection analyzer (Lachat Instruments, Inc., Milwaukee, WI, USA). Lignin was analysed using a modified acetyl-bromide method and values were compared with an NIST certified pine standard (Iiyama & Wallis 1990). To validate that core rotation did exclude hyphae, hyphal lengths were measured on the surface of the litterbags in a manner similar to the inserted membrane technique developed by Balaz & Vosatka (2001). Hyphal length was measured in 20 haphazardly located fields on each litterbag at 50 × magnification on a dissecting microscope using the gridline intersect method (Giovannetti & Mosse 1980). Remaining C, remaining N and $\delta^{15}N$ from each harvest were analysed with a two-factor ANOVA with litter type (EM vs. NM) and hyphal ingrowth (+H vs. −H) and the interaction term as factors. We performed t-tests on soil moisture and hyphal length measurements (α = 0.05 for all tests).

RESULTS

Ectomycorrhizal roots from piñon pine decomposed much more slowly than NM roots of the same size class (ANOVA, tissue type, $P < 0.05$ at 10 and 24 months; Fig. 2a). After 2 years of field decomposition, NM roots lost 19.3% of their initial C mass, whereas EM roots lost only 7.0%. EM roots, which began with much higher N concentration (Table 1), immobilized an additional 15% while NM roots released 5% of their initial N content (ANOVA, hyphal ingrowth, $P < 0.01$ for all dates; Fig. 2b).

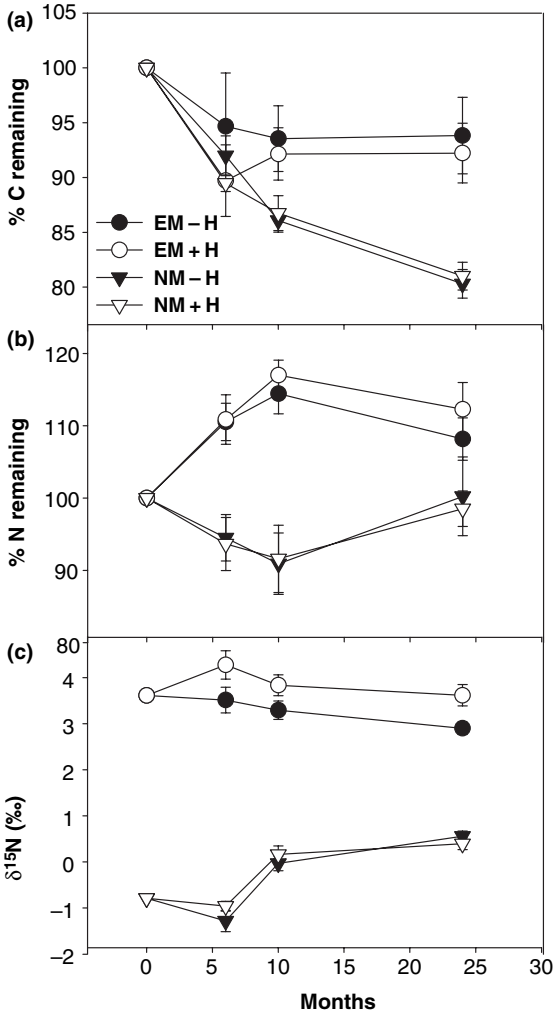

Figure 2 Carbon and nitrogen dynamics in ectomycorrhizal (EM) and non-mycorrhizal (NM) litter throughout 2 years of decomposition in unturned cores which allowed hyphal ingrowth (EM ○; NM ▽) and turned cores which excluded ingrowing hyphae (EM ●; NM ▼) summarized as means ± SE (n = 7–8). (a) The % of initial C mass remaining in each litter type. EM roots lost only one-third as much C as NM roots and lost no C mass during the final 14 months. (b) The % of initial N mass remaining in each litter type. EM roots, which began with higher initial [N], immobilized N for the first 10 months of decomposition while NM litter released N. (c) Natural abundance of ^{15}N in decomposing litter. EM roots begin with higher ^{15}N because of natural fungal enrichment. A consistent 1‰ enrichment indicates altered turnover of N in EM litters exposed to ingrowing hyphae.

Table 1 Initial chemistry of piñon ectomycorrhizal (EM) and non-mycorrhizal (NM) root litter

	EM	NM
% N	1.57 ± 0.08	0.71 ± 0.03
C : N	28.6 ± 1.0	65.5 ± 2.4
% Lignin	12.3 ± 1.3	13.8 ± 3.4
% P	0.12 ± 0.01	0.08 ± 0.01

The presence of live hyphae had no influence on the masses of C and N remaining in litter ($P > 0.05$ for all dates; Fig. 2a, b). Hyphal ingrowth did generate slight but consistent trends of greater C loss and greater N immobilization in EM litter in unturned +H cores. Hyphal exclusion did affect isotopic composition of litter (ANOVA, hyphal ingrowth, $P < 0.05$ at 24 months; Fig. 2c). Hyphal ingrowth caused a *c.* 1‰ enrichment of $\delta^{15}N$ in EM litter but not in NM litter resulting in a significant interaction between hyphal ingrowth and litter type (ANOVA, litter type × hyphal ingrowth interaction, $P < 0.05$ at 24 months; Fig. 2c). The hyphal lengths measured in +H cores were four times greater than in −H cores, verifying that core rotation successfully excluded most fungal ingrowth (*t*-test, $P < 0.05$, data not shown). Core rotation did not alter gravimetric soil moisture inside the cores (*t*-test, $P > 0.05$).

DISCUSSION

We found that root litter type (EM vs. NM) drove C and N dynamics throughout decomposition. The 65% depression of decomposition rates resulting from EM colonization is comparable with the difference in decomposition rates between roots of trees and roots of herbaceous plants (Silver & Miya 2001). Moreover, though it has much higher initial [N], EM litter immobilized an additional 15% of its initial N in the first 10 months of the study period while NM litter exhibited a net release over the same time period (Fig. 2b). Though EM litters represent a large flux of N-rich matter into soil, that litter N appears to remain unavailable to plants and soil organisms.

Hyphal ingrowth had no effect on C or N mass remaining in either litter type (Fig. 2a, b) but sharply increased the $\delta^{15}N$ of EM litter (Fig. 2c), suggesting altered turnover of that N pool. Mycorrhizal fungi are strongly enriched in $\delta^{15}N$ (Hobbie *et al.* 2001), even in comparison with saprotrophic fungi from the same ecosystem (Henn & Chapela 2001).

Simultaneous enhancements of gross N mineralization and N immobilization by ^{15}N-enriched, ingrowing mycorrhizal hyphae perhaps best explain this effect. The potential for mycorrhizal fungi to short-circuit the N cycle by recycling their own N in this manner has been demonstrated in the laboratory (Kerley & Read 1998; Lindahl *et al.* 2002). Differences in N processing rates may indicate that live EM fungi gain primary access to the N in their own decomposing litter. Still, after 2 years, hyphal ingrowth did not strongly influence net C or N dynamics, and accordingly, we conclude that the afterlife effects of EM fungi on root litter quality supercede the effects of live EM fungi on root litter decomposition in this ecosystem.

From litter to soil organic matter

Studies of aboveground plant litter have consistently found that tissues with high [N], or low C : N ratios, tend to decompose more rapidly (Melillo *et al.* 1982). These indices of litter quality do not appear to predict root decomposition in the same way. Despite having initial C : N ratios of 29 compared with 66 for NM roots (Table 1), EM roots decomposed much more slowly than NM roots, likely owing to fundamental differences in carbon chemistry between fungal and plant tissues. The primary structural polymer in fungi is chitin, which is N-rich (7% N by mass) but resists decomposition (Swift *et al.* 1979). The surprising recalcitrance of EM roots could explain anomalous trends among root decomposition studies such as a strong negative relationship between [N] and decomposition rate in members of Pinaceae (summarized as conifers in Silver & Miya 2001) which commonly host EM fungi.

Another controversial precept of soil ecology is that stable soil organic matter largely arises from recalcitrant plant compounds such as lignin. However, recent molecular analyses employing isotopic tracers to age carbon found no evidence that lignin contributes directly to old soil organic matter (Mikutta *et al.* 2006). Instead, the oldest constituents of soil may derive from N-containing compounds including chitin (Gleixner *et al.* 2002). We suggest that the slow turnover of N-rich fungal tissues, which may become physically fractured but resist chemical decomposition, could contribute directly to the formation of stable soil organic matter, which also has high N content. In light of the observation that EM abundance generally correlates with organic soil accumulation (Read 1991), our findings support the prospect that EM fungi not only proliferate in stable, organic soils but may also contribute to their formation and maintenance.

It is known that plants species may drive soil properties such as N availability (Chapman *et al.* 2006). Mycorrhizal type may be one characteristic that mediates unique species effects. Despite initial N concentrations twice those found in NM litter, EM root litter immobilized much more N in the early stages of decomposition. EM litter represents a large pool of organic N that mineralizes slowly and so, could hold importance for N retention in N-limited ecosystems. Field estimates of mycorrhizal hyphal decomposition rates are required to constrain the total contribution of mycorrhizal litters to biogeochemical cycles.

Historically, the live mycorrhizal function as a decomposer was recognized as the primary mechanism by which mycorrhizal fungi may impart an influence on ecosystem processes such as decomposition (Gadgil & Gadgil 1971). Recent acknowledgement of the large amount of C allocated to, and ultimately deposited by, mycorrhizal fungi has spurred debate regarding the residence time of senesced mycorrhizal structures in soils, most of which focuses on arbuscular mycorrhizas (Langley & Hungate 2003; Staddon *et al.* 2003; Steinberg & Rillig 2003; Zhu & Miller 2003; Olsson & Johnson 2005). We have shown here that EM root litter decomposes much more slowly than NM roots in piñon pine. The influence of mycorrhizal colonization on ecosystem properties of the piñon woodland appeared to outweigh the influence of live fungal activity. Therefore, we believe that mycorrhizal influence on resource cycles could be mediated directly by the turnover of dead fungal products rather than solely through the impact of live mycorrhizae on nutrient absorption and decomposition. Understanding these effects will expose the black box of belowground C and N cycling and perhaps inform global change mitigation strategies.

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