Fine roots and mycorrhizal fungi accelerate leaf litter decomposition in a northern hardwood forest regardless of dominant tree mycorrhizal associations

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Heading: Both AM- and ECM-associated fine roots stimulate litter decay in northeastern U.S. forests

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Summary

- Fine roots and mycorrhizal fungi may either stimulate leaf litter decomposition by providing free-living decomposers with root-derived carbon, or may slow decomposition through nutrient competition between mycorrhizal and saprotrophic fungi.
- We reduced the presence of fine roots and their associated mycorrhizal fungi in a northern hardwood forest in New Hampshire, USA by soil trenching. Plots spanned a mycorrhizal gradient from 96% arbuscular mycorrhizal (AM) associations to 100% ectomycorrhizal (ECM)-associated tree basal area. We incubated four species of leaf

litter within these plots in areas with reduced access to roots and mycorrhizal fungi and in adjacent areas with intact roots and mycorrhizal fungi.

- Over a period of 608 days, we found that litter decayed more rapidly in the presence of fine roots and mycorrhizal hyphae regardless of the dominant tree mycorrhizal association. Root and mycorrhizal exclusion reduced the activity of acid phosphatase on decomposing litter.
- Our results indicate that both AM- and ECM-associated fine roots stimulate litter decomposition in this system. These findings suggest that the effect of fine roots and mycorrhizal fungi on litter decay in a particular ecosystem likely depends on whether interactions between mycorrhizal roots and saprotrophic fungi are antagonistic or facilitative.

Key words: Litter decomposition, fine roots, mycorrhizal fungi, Gadgil effect, extracellular enzymes

Introduction

Forests are one of the largest terrestrial carbon (C) sinks, and nearly half of the C stored in forests is contained in litter and soil (Pan *et al.*, 2011). Leaf litter decomposition is one of the primary mechanisms by which organic matter enters this soil pool (Aber & Melillo, 1980). While litter decomposition is primarily regulated by litter chemistry and climate (Aerts, 1997; Trofymow *et al.*, 2002; Zhang *et al.*, 2008; Tuomi *et al.*, 2009), soil microbial community composition is increasingly recognized as an important factor in leaf litter decay rate (McGuire & Treseder, 2010; Wieder *et al.*, 2015; Sulman *et al.*, 2017; Bradford *et al.*, 2017). A higher functional diversity (Maron *et al.*, 2018), and abundance (Heijboer *et al.*, 2018) of microbes can accelerate organic matter decomposition, and a species-rich microbial community is more likely to contain the functional groups necessary to decompose the variety of compounds in complex litter material (Schneider *et al.*, 2012).

In addition to free-living bacteria and fungi, mycorrhizal fungi often play a key role in the litter decomposition process. Mycorrhizal fungi may accelerate decomposition directly, through the exudation of enzymes, or indirectly by stimulating the free-living microbial community with carbon-rich root exudates (Talbot *et al.*, 2008; Bengtson *et al.*, 2012; Paterson *et al.*, 2016; Zhou *et al.*, 2019). Conversely, resource competition between mycorrhizal fungi and other microbial groups may slow litter decomposition rates by suppressing decomposer activity (Gadgil & Gadgil, 1971; Bending, 2003; Fernandez & Kennedy, 2015; Brzostek *et al.*, 2015).

Virtually all tree species form associations with either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi. Evidence from forests around the world suggests that the relative dominance of these fungal groups influences biogeochemical processes including litter decomposition (Read & Perez-Moreno, 2003; Phillips *et al.*, 2013; Frey, 2019). However, the nature of these effects is highly variable across studies: both AM and ECM fungi have been shown to accelerate litter decomposition in some studies, and inhibit it in others. ECM fungi may accelerate decomposition using extracellular enzymes and other oxidative decay mechanisms

retained from their saprotrophic ancestors (Lindahl & Tunlid, 2015; Shah *et al.*, 2016; Nicolás *et al.*, 2019). However, this effect is thought to be stronger for the decomposition of soil organic matter rather than fresh litter (Sterkenburg *et al.*, 2018), perhaps owing to the limited capacity of ECM fungi to produce cellulolytic enzymes (Pellitier & Zak, 2018) or the vertical separation of ECM fungi from the saprotroph-dominated litter layer (Lindahl *et al.*, 2007). Additionally, AM fungi have been shown to promote litter decay in laboratory studies, though it is unclear whether this effect is due to changes in the composition or activity of the free-living soil microbial community or the activity of the AM fungi themselves (Gui *et al.*, 2017; Xu *et al.*, 2018). Both AM and ECM fungi are known to release plant-derived C into the mycorrhizosphere (Herman *et al.*, 2012; Yin *et al.*, 2013; Gorka *et al.*, 2019), which may prime the activity of free-living bacteria and fungi and indirectly stimulate litter decomposition (Hodge *et al.*, 2001; Bunn *et al.*, 2019).

Both ECM and AM fungi have also been observed to suppress litter decomposition rates. In what is perhaps the best-known effect of mycorrhizal fungi on litter decay, ECM fungi may inhibit litter decomposition by outcompeting free-living fungi for limiting nutrients and reducing their ability to degrade organic C (Gadgil & Gadgil, 1971; Fernandez & Kennedy, 2015; Bödeker *et al.*, 2016; Sterkenburg *et al.*, 2018). This process, known as the "Gadgil effect" has been studied in forests around the world, and has been proposed as a mechanism by which ECM-dominated forests may contain larger stores of soil C than their AM-dominated counterparts (Averill *et al.*, 2014). Though less common, some studies suggest that AM roots and fungi may also suppress litter decomposition (Brzostek *et al.*, 2015) and inhibit the growth of other soil microbes (Filion *et al.*, 1999; Welc *et al.*, 2010) by competing with free-living microbes for limiting nutrients.

The wide range of effects observed for both AM and ECM fungi on litter decomposition may be due in part to differences in the chemistry of the litter used in these studies and the soil conditions in which the litter was incubated (Fernandez *et al.*, 2019). A root and mycorrhizal priming effect on decomposition, wherein mycorrhizosphere bacteria and fungi are stimulated by hyphal transfer and exudation of root-derived C, should be strongest when the litter substrate available to microbes is difficult to degrade: the sudden release of these microbes from an energy-limited state may result in a sharp increase in decomposition rate. Further, competition for nutrients between ECM and saprotrophic fungi, as posited by literature on the Gadgil effect, is unlikely unless the microbial community is strongly nutrient limited. These conditions are often found in conifer forests, where needle litter and soil have a high ratio of carbon to nitrogen (C:N), inducing the necessary N competition between microbial groups that leads to slower litter decay (Smith & Wan, 2019). In fact, almost all demonstrations of the Gadgil effect to date have taken place in pine-dominated forests using pine needles as the litter substrate (Fernandez & Kennedy, 2015; Sterkenburg *et al.*, 2018; Fernandez *et al.*, 2019). However, it is unclear whether this microbial competition is due to the specific microbial groups found in these forests, the nutrient content of the litter substrate, or other associated conditions of the soil and climate common in pine-dominated ecosystems. Therefore, tests in deciduous forests with multiple litter

species are necessary to disentangle the effects of soil chemistry, microbial community, and litter chemistry on the presence and strength of the Gadgil effect.

Most studies of the effects of mycorrhizal fungi on litter decay have been conducted in ecosystems dominated by a single mycorrhizal type, yet many forests contain both AM-associated and ECM-associated tree species (Phillips *et al.*, 2013; Steidinger *et al.*, 2019). In isolating AM and ECM-associated forest types, it is unclear whether mechanisms explored in these studies represent conditions found in mixed forests, where interactions between AM and ECM fungi may counteract their individual effects on the free-living fungal community. For example, suppression of saprotrophic activity posited by the Gadgil effect, while plausible in N-limited forests dominated by ECM fungi, could be offset in mixed forests by C exudation from AM roots and fungal hyphae (Kaiser *et al.*, 2015), which can stimulate mycorrhizosphere bacteria and fungi (Talbot *et al.*, 2008; Phillips *et al.*, 2012; Herman *et al.*, 2012; Nuccio *et al.*, 2013).

These observations led us to ask how mycorrhizal fungi influence leaf litter decomposition in a temperate deciduous forest, where root-microbial interactions may result in either a priming effect (accelerating litter decay) or a Gadgil effect (suppressing litter decay). Taking advantage of a natural gradient of mycorrhizal type in a forest with both AM- and ECM-associated tree species, we compared decay rates of litter incubated in undisturbed soil to litter incubated with reduced access to roots and mycorrhizal hyphae within a series of plots ranging from 100% ECM-associated tree species to 96% AM-associated tree species.

We expected that excluding ECM-associated roots would accelerate litter decomposition by releasing free-living decomposers from competition with ECM fungi, while excluding AM-associated roots would slow litter decomposition by reducing labile C inputs from root exudates, a primary source of energy for soil microbes. We also hypothesized that the effects of excluding mycorrhizal fungi would be stronger for litter with higher C:N, both because ECM-saprotroph competition should be strongest when N is more limited, and because reducing root C exudates should make relatively nutrient-poor litter material energetically unfavorable to decomposers.

Materials and Methods

Site description

We established our study plots in November 2017 at Hubbard Brook Experimental Forest in Woodstock, NH (43°56′N, 71°46′W). The forest is composed of typical northern hardwood species, including sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britton) and American beech (*Fagus grandifolia* Ehrh.), with smaller quantities of paper birch (*Betula papyrifera* Marsh.), Eastern hemlock (*Tsuga canadensis* L. Carrière), white ash (*Fraxinus americana* L.), and red maple (*Acer rubrum* L.) The forest is underlain by rocky spodosols with well-developed horizons and a thick (3-10 cm) litter layer. Within this forest, we

established twelve plots that varied in their relative abundance of AM and ECM-associated tree species, from 96% AM-associated to 100% ECM-associated trees as measured by basal area. Plot locations were determined based on mycorrhizal associations of the tree species present in the study area and on the soil suitability; a plot was established only when three one-square meter subplots could be identified with no large rocks or roots to a depth of 30 cm, allowing for our trenching procedure. The perimeter of each plot was drawn to accommodate the locations of the interior subplots, with a five meter radius extending from each subplot edge into the surrounding forest to capture the relative abundance of neighboring tree species. All plots were located within a small (2.4 ha) area of forest to minimize differences in microclimate and topography.

Subplot treatments

Within each study plot, we randomly assigned one treatment to each of three subplots: root and mycorrhizal exclusion (hereafter "trenched"), disturbance control (hereafter "disturbed"), and control (Figure 1). Trenched subplots were isolated from the surrounding soil by cutting through the soil around the subplot perimeter and inserting four plexiglass walls around the edges to a depth of 30 cm. We chose 30 cm as the depth of our trenches based on reports from a nearby study area that ~70% of fine roots (0-2 mm) are found within the top 25 cm of the soil profile, and that at Hubbard Brook, the median fine root depth is ~11 cm (Yanai *et al.*, 2008). At the subplot corners, plexiglass sheets were fitted into notched wooden stakes to prevent root ingrowth into the interior soil. Disturbed subplots were cut to a depth of 30 cm but no barriers were inserted, allowing us to test for unintended effects of the trenching procedure on decay rates. Control subplots were marked with flags, but left intact and undisturbed.

Soil physical and chemical properties

Immediately prior to trenching, we collected three soil samples in random locations within each subplot with a soil corer (5 cm diameter). From these samples, we removed all fine roots and sorted them by mycorrhizal type based on visual identification of ECM colonization and other morphological features (Yanai *et al.*, 2008). Following identification, roots were oven dried at 60 °C. We measured extractable nitrate and ammonium from the remaining soil from these samples with an Astoria-Pacific Discrete Analyzer (Astoria-Pacific, Inc., Clackamas, OR, USA). To confirm that the tree species and root biomass in our study plots corresponded to differences in mycorrhizal communities, we estimated the relative abundance of AM fungi in soil collected within control subplots using phospholipid fatty acid (PLFA) analysis by gas chromatography mass spectroscopy (Microbial ID, Inc. Newark, DE, USA). Soil was sampled from the top 5 cm of the organic horizon in September 2019 and kept frozen until processing within a week of collection using standard methods (Buyer & Sasser, 2012). Peaks for AM fungi (16:1ω5) were identified using the Sherlock System (MIDI, Inc., Newark, DE, USA). To investigate whether our exclusion treatment resulted in unintentional changes to soil moisture, we measured soil volumetric water content in each subplot within three plots using moisture sensors buried 5 cm

below the surface of the litter layer (EC-5 VWC Sensor, Model E-240-40593, Decagon devices, Pullman, WA, USA). Soil moisture was logged every hour by a datalogger (HOBO H21-USB Micro Station, Onset, Bourne, MA).

Litter decomposition

We created mesh litter bags with 2-mm fiberglass window screen and filled each bag with 1 g (3-5 leaves) of air-dried litter from a single tree species. We collected litter from the four most common tree species in our study area: two AM-associated species (*A. saccharum* and *F. americana*) and two ECM-associated species (*F. grandifolia* and *B. alleghaniensis*). These species span a wide range of nitrogen and lignin content, allowing us to assess the sensitivity of decay rates to litter chemistry. Leaves were collected in Fall 2016 from the same forest area where the incubation took place using baskets lined with mesh. Leaves were collected before rainfall events to reduce leaching losses and were immediately brought to the lab and air-dried in paper bags until use. Dried leaves were sorted by species and litter from all collection baskets was aggregated to form one homogenized sample per species. Samples of air-dried litter from each species were oven dried to determine initial moisture content and subsequently ground for analysis of total C and N (Carlo- Erba Instruments, Wigan, UK), and lignin, cellulose, and hemicellulose content (ANKOM A2000 Digestion Unit, ANKOM Technology, Macedon, NY, USA). Leaves chosen for the litter bags were visually screened and those with signs of herbivory or pathogens were excluded. Three bags of each species were placed at the surface of the litter layer in each experimental subplot in November 2017, with bag placement determined randomly within the subplots. After 186, 278, and 608 days, one bag of each species' litter was retrieved from each subplot. Bags were carefully brushed to remove adhering litter, fine roots, and soil on the exterior of the mesh, placed in plastic bags, and transported back to the lab in coolers with ice. The remaining litter material was carefully removed from the mesh bags, cleaned, weighed, and dried at 60°C. On the first and last collection dates, subsamples of litter material were isolated for enzyme analysis and frozen within 48 hours of sampling.

Extracellular enzyme activity

To assess whether differences in decomposition rate were explained by differences in enzyme activity on the litter, we measured the potential activities of six extracellular enzymes on litter material from litter bags collected in May 2018 (after 186 days) and July 2019 (after 608 days). Only litter incubated in trenched and control subplots was used for enzyme analyses. Litter was handled with tweezers or gloved hands at all times, and subsamples were collected from a minimum of three areas of the litter sample within each mesh bag to capture the heterogeneity of the litter material. These small sections of litter material from each sample (50 mg total) were then pooled for analysis. After the first collection, we determined the potential activities of three hydrolytic enzymes (β-glucosidase: BG, acid phosphatase: AP, and β-N-acetylglucosaminidase: NAG) and two oxidative enzymes (polyphenol oxidase: PPO, and peroxidase: PER) on white ash and American beech litter collected from four representative plots along our mycorrhizal

gradient. For samples collected in July 2019, we additionally measured cellobiohydrolase (CBH) activity and conducted assays on all four litter species in all twelve plots. These enzymes were chosen to represent the activity of microbes targeting labile carbon substrates, such as sugars and cellulose (BG, CBH), phosphates (AP), chitin and peptidoglycan (NAG; Caldwell, 2005), and complex macromolecules like phenolic compounds and lignin (PPO, PER; Burns *et al.*, 2013). Potential enzyme activities were calculated using established methods (Saiya-Cork *et al.*, 2002); briefly, litter samples were homogenized in a sodium acetate buffer solution with a tissue homogenizer and incubated with either methylumbelliferone-linked substrates (for hydrolytic enzymes) or L-dihydroxyphenylalanine (for oxidative enzymes) in a 96-well plate for either 30 minutes (NAG, AP), 2 hours (BG, CBH) or 24 hours (PPO and PER), after which fluorescence or absorbance was determined with a plate reader (Tecan Group Ltd., Zurich, Switzerland). Potential enzyme activities were calculated as described in Saiya-Cork *et al.* (2002).

Data analysis

Decomposition rate

We calculated the initial decay constant, *k*, by fitting a single-pool decomposition function to model the change in mass over time for each litter species within each subplot. Larger values of *k* indicate faster litter decomposition. We estimated the decay constant with non-linear regression using the `nlme` package in R (Harmon *et al.*, 2009; Pinheiro *et al.*, 2020). A single-pool model was chosen for these data after double-pool decomposition models failed to converge within biologically realistic parameters. Given the relatively short duration of this incubation and the climate of the study area, a single-pool model best captures the early stage of decomposition that our litter samples were likely to experience during the study.

We evaluated hypotheses regarding litter decay rate with a linear mixed effects model that included litter species and trenching treatment as categorical fixed effects, and the proportion of tree basal area associated with ECM fungi as a continuous variable. We also evaluated interactions between mycorrhizal dominance and treatment, and between litter species and treatment. We included plot (n=12) as a random effect to account for plot-level variation in soil conditions that may have influenced decay. After estimating parameters with the full model, nonsignificant interaction effects were removed and the model was re-evaluated. We then used a post-hoc multiple comparisons test (Tukey's HSD) to assess differences between the decay rate of different litter species and litter incubated in the different root exclusion treatments.

We tested whether the trenching treatment affected soil moisture (a possible confounding effect) through analysis of three plots where soil moisture was measured continuously. We constructed generalized least squares models for each plot using daily average soil moisture in each subplot. We included trenching treatment as a fixed effect and added a temporal autocorrelation structure to address the co-dependence of measurements taken closely in time. We then tested whether the variation in mean subplot moisture within these three plots affected litter decay rate using a

linear mixed effects model with mean soil moisture as a fixed effect and litter species as a random effect.

Soil conditions along the mycorrhizal gradient

We assessed how soil nitrogen concentrations, fine root biomass, and the abundance of AM fungal biomarkers varied along the mycorrhizal gradient using simple linear models. Measurements of root abundance and soil N concentration collected from each subplot before the trenching treatment were averaged within each plot and transformed (natural log) when necessary to meet assumptions of linear regression. Two extreme outliers (> 5 standard deviations above mean concentration) were removed from analyses of nitrate and ammonium.

Extracellular enzyme activities

We evaluated patterns in enzyme activity with linear mixed effects models that included litter species, trenching treatment, plot mycorrhizal type, trenching x dominant mycorrhizal type, and plot as a random effect.

Results

Litter decomposition rate

Trenching reduced the decay rate (k) of all species of litter relative to litter incubated in control subplots (mean $k_{trenched}$= 0.586, mean $k_{control}$= 0.650; $F_{2,125}$= 5.95, p= 0.003; Figure 2a), a difference equivalent to a 62 day increase in mean litter residence time ($1/k$). This reduction in litter decay with trenching was consistent regardless of the mycorrhizal association of trees in the plots ($F_{2,117}$= 1.02, p= 0.36 for interaction; Figure 3).

The average litter decomposition rate in the disturbed plots fell between those of the trenched plots and control plots (mean $k_{disturbed}$= 0.617) , and there was no statistically significant difference in k between disturbed and trenched plots or disturbed and control plots (Table 1). Accordingly, the disturbed plots were excluded from subsequent analyses of litter enzyme activity and soil microbial community composition.

The strength of the root exclusion effect did not vary with litter species ($F_{6, 117}$= 1.31, p= 0.26 for interaction). Overall, the average litter decay rate varied by species in the order *F. grandifolia* < *A. saccharum* < *B. alleghaniensis* < *F. americana* (Figure 2b; $F_{3, 125}$= 115.2, p< 0.0001). After 608 days, the mass of remaining litter from *F. grandifolia* was ~60% of initial, compared to ~40% of initial mass for all other species (Figure 4). These patterns corresponded loosely with litter lignin concentration, which varied from an average of 12.6% (*F. grandifolia*) to 8.9% (*F. americana*; Table S1).

Litter decomposition rates were unrelated to the mycorrhizal type of the dominant trees in each plot ($F_{1,10}$= 0.002, p= 0.97; Table 1).

Soil conditions along the mycorrhizal gradient

The proportion of fine root biomass from ECM-associated tree species increased with percent ECM tree basal area in each plot ($F_{1,10}$= 21.71, p= 0.0009, r^2= 0.65; Figure S1a), and the relative proportion of AM fungal biomarkers in the microbial community was negatively associated with increasing percent ECM tree basal area ($F_{1,10}$= 3.97, p= 0.07, r^2= 0.28; Figure S1b). Total fine root biomass had a weak negative relationship with percent ECM tree basal area ($F_{1,10}$= 2.79, p= 0.13; Table S2).

Average initial nitrate concentrations in each plot ranged from 0.044 to 3.55 mg per kg of dry soil, and ammonium concentrations ranged from 16.4 to 95.0 mg per kg of dry soil (Table S2). Average nitrate and ammonium concentrations did not vary with plot mycorrhizal association (nitrate: $F_{1,10}$= 2.37, p= 0.15; ammonium: $F_{1,10}$= 0.52, p= 0.49).

Treatment effects on soil moisture

Trenching had no consistent effect on soil moisture in the three plots where continuous measurements were made over the course of the study; in plot 10, the trenching treatment was unrelated to soil moisture, in plot 7, the trenched subplot was on average 0.035 m^3m^{-3} (9.1 %) drier than the control subplot (t= -4.61, p <0.001), and in plot 9, the trenched subplot was 0.029 m^3m^{-3} (11.3%) drier than the control subplot (t= -2.99, p= 0.003; Figure S2a). The soil moisture in disturbed subplots did not consistently rank higher or lower than that of trenched or control subplots. Further, the rate of litter decomposition in these plots was not affected by the average soil moisture during the study period ($F_{1,31}$ = 1.07, p= 0.309; Figure S2b).

Extracellular enzyme activity on leaf litter

After 608 days, BG, CBH, NAG, and PPO activities increased with ECM tree basal area in trenched subplots relative to control subplots (Table S3; *see interaction between treatment and mycorrhizal dominance*). The activity of AP was significantly lower on litter in trenched subplots at the end of the 608-day study period ($F_{1,78}$= 6.22, p= 0.015; Table S3), but this effect was not dependent on plot mycorrhizal type ($F_{1, 78}$= 0.026, p= 0.87; Figure 5).

At the time of the first litter collection (186 d), all enzyme activities excluding peroxidase were higher on white ash litter than American beech litter (all p values < 0.05), but after 608 days, litter species had only weak effects on the activity of BG, CBH, and PER ($F_{3,79}$ < 2.70, p > 0.05; Table S3).

Discussion

Effects of fine root and mycorrhizal exclusion on litter decomposition rate

Contrary to our hypothesis that the exclusion of AM and ECM roots would have divergent effects on decomposition, we found that excluding both AM and ECM roots and fungi reduced the decomposition rate of litter material. Though we cannot distinguish the effects of mycorrhizal fungi from those of fine roots alone, the lack of a trend along our mycorrhizal gradient suggests that fine roots associated with AM or ECM fungi have similar, positive effects on litter decay in this system. While this pattern is in contrast to the Gadgil effect sometimes noted in ECM-dominated systems, it supports the large body of literature showing a stimulatory effect of fine roots on microbial activity and organic matter decomposition (Zhu & Ehrenfeld, 1996; Hodge *et al.*, 2001; Herman *et al.*, 2012; Jackson *et al.*, 2019; Adamczyk *et al.*, 2019; Han *et al.*, 2020), which can lead to faster rates of surface litter decomposition (Zhu & Ehrenfeld, 1996; Moore *et al.*, 2019). Though some have reported that vertical separation of mycorrhizal and saprotrophic fungi suggests that mycorrhizal fungi may not exploit surface litter as an important nutrient source (Lindahl *et al.*, 2007), we saw ample evidence of both AM and ECM-associated fine roots growing into the litter within our mesh decomposition bags. This observation, coupled with the similar effects of AM and ECM-associated roots on litter decay rate, suggests that both AM and ECM-associated fine roots can exploit surface leaf litter as a nutrient source (Bending, 2003; Bunn *et al.*, 2019). However, an alternative explanation for the reduction in litter decay with trenching may be that the presence of newly severed roots resulted in microbial activity shifting toward decomposing fine root litter rather than leaf litter. Although roots tend to have relatively low nutrient and high lignin content relative to leaves (Hobbie *et al.*, 2010), and thus may not be preferable as a decomposition substrate, the soluble carbon (i.e., sugars and other root exudates) contained in fine roots may have stimulated fine root decay at the expense of litter decay. However, it is unlikely that decaying roots had more than a modest effect on litter decomposition in this study given that the mean decomposition constant in disturbed subplots was indistinguishable from that of control subplots.

Further, we did not find that reducing access to roots and mycorrhizal fungi had a stronger effect on litter with a high C:N; instead, all four litter species responded similarly to trenching. As has recently been reported in pine and oak-dominated forests (Fernandez *et al.*, 2019), we expected stronger competition between mycorrhizal fungi and decomposers in plots with lower soil N concentrations and litter with high C:N and thus a more marked response to mycorrhizal exclusion. We expected the same pattern in the event of a root priming effect on litter decay, where trenching should have a stronger effect on the decomposition of litter with higher C:N because decomposer activity may be more dependent on root C subsidies to break down complex litter material. Though other facets of litter chemistry, including the relative proportion of cellulose, hemicellulose, and lignin, are likely to influence litter decay rates, litter C:N indicates the suitability of the litter as a nitrogen source for microbes. We suggest that litter C:N did not

affect the strength of the trenching effect because N content was not low enough in any of the four litter species to either induce competition between mycorrhizal and saprotrophic fungi or result in differences in the importance of root C exudates for decomposer activity (Smith & Peay, 2020). This explanation is supported by recent work indicating that environmental context, particularly the severity of N limitation, determines whether AM or ECM fungi significantly influence organic matter decomposition in a particular ecosystem (Smith & Wan, 2019; Fernandez *et al.*, 2019; Zhou *et al.*, 2019). The similar responses of litter decay to trenching despite differences in root mycorrhizal type and litter chemistry suggests that the presence of fine roots and mycorrhizal hyphae stimulates leaf litter decomposition and is no more effective for litter with relatively low N content.

Changes in litter enzyme activity with root and mycorrhizal exclusion

We expected that reducing fine roots and mycorrhizal hyphae would reduce mycorrhizal biomass and C subsidies to rhizosphere microbial communities (Högberg *et al.*, 2001; Kaiser *et al.*, 2015; Paterson *et al.*, 2016), resulting in lower enzyme activity on decomposing litter (Sterkenburg *et al.*, 2018; Moore *et al.*, 2019). Instead, we found that acid phosphatase (AP) was the only enzyme reduced by trenching as measured at the end of the experiment. This pattern may suggest that AP is produced primarily by fine roots or mycorrhizal fungi, or that AP activity is indirectly stimulated by living roots and mycorrhizal hyphae. Because of its role in liberating phosphorus, a limiting nutrient in this ecosystem (Fisk *et al.*, 2014; Goswami *et al.*, 2018), increases in AP activity in the presence of active roots and mycorrhizal hyphae may be critical for plant productivity in northern temperate forests. However, given that trenching reduced the activity of only one enzyme we measured, and that this measurement represents only one point in time, lower enzyme activity was unlikely to be the explanation for the slowed decay of litter incubated in trenched soil in this study.

Interestingly, we saw that C- and N-targeting enzyme activities responded to the mycorrhizal gradient only on litter in trenched subplots, where reducing ECM-associated roots resulted in higher rates of BG, CBH, NAG, and PPO activity. This pattern suggests a process similar to the Gadgil effect, where free-living microbial enzyme activity may have been suppressed in the presence of ECM-colonized roots. In this case, resource competition on decaying litter may result in shifts toward higher abundances or activity of ECM fungi compared with decomposers, but if so, these shifts seem unrelated to the rate of overall litter mass loss.

Extracellular enzyme activity on litter varied strongly with litter species in the early stage of decomposition, but this effect was dampened after 608 days of litter incubation, perhaps because microbial colonizers had completely exploited the most labile material and begun decomposing structural C and other complex molecules that are equally recalcitrant across litter species (Schneider *et al.*, 2012). These patterns suggest that enzyme activity on decaying litter, though indicative of the sometimes rate-limiting depolymerization stage of organic matter decomposition (Schimel & Bennett, 2004), does not correspond well with rates of litter mass loss.

Challenges with soil trenching procedures

Despite its widespread use in ecosystem studies, soil trenching can induce unintentional changes in soil moisture and microbial activity in soil (Savage *et al.*, 2018). We aimed to account for such artifacts, including an initial pulse of root necromass and less water uptake from living roots, by creating disturbed subplots within each study plot, cut to the same depth as the trenched plots but not isolated with an impermeable barrier so that roots could re-colonize the area. Given the relatively short timeframe of the experiment and the fact that artifacts from soil trenching can persist for several years (Savage *et al.*, 2018), it is likely that soil conditions in our disturbed subplots were similar to those of the trenched subplots, particularly at the beginning of the litter incubation. However, our continuous measurements of soil moisture showed idiosyncratic effects of trenching on moisture dynamics over time, with no clear differences between subplot treatments (Figure S2a), and in plots where we measured soil moisture continuously, the decomposition constant was unrelated to soil moisture (Figure S2b). Additional studies at Hubbard Brook Experimental Forest and Harvard Forest in Massachusetts show that microbial activity, measured as soil respiration, is resistant to variation in soil moisture of the magnitude that we observed in our continuously monitored plots, especially given their relatively high moisture levels (~20-30% v/v; Savage & Davidson, 2001; Lang *et al.*, 2019). Therefore, we suggest that the activity of decomposers in this system was unlikely to be limited by soil moisture during our study period, and that soil moisture was unlikely to influence litter decomposition rate.

Another potential artifact of soil trenching is the accumulation of mineral N following reduced root N uptake. This change will likely result in greater N availability to microbes and thus lower rates of microbial activity and decomposition (Freedman *et al.*, 2016; Entwistle *et al.*, 2018; Zak *et al.*, 2019; Argiroff *et al.*, 2019). We measured mineral N only at the start of the experiment, to capture initial conditions in our study plots, so we cannot rule out the possibility that our trenching treatment increased N availability to microbes, reducing the need to decompose organic matter for N and resulting in lower litter decay rates. However, if this were the mechanism behind the observed decrease in decomposition rate in trenched plots, it is surprising that the strength of this effect did not vary with plot mycorrhizal type. Given their differing abilities to utilize organic N substrates, the free-living microbial communities associated with AM and ECM fungi may be expected to respond differently to soil N availability (Midgley & Phillips, 2014), with AM-associated microbial communities adapted to take up mineral N more efficiently than fungal-dominated ECM communities that specialize on organic forms of N. Thus, we might have expected AM-dominated plots to respond more strongly to a change in mineral N availability, yet we see no interaction between the trenching treatment and the mycorrhizal dominance in each plot.

Conclusions

We show that reducing access to roots and mycorrhizal fungi during the decomposition process slowed the decay of leaf litter from four common species in a northern hardwood forest. Further, we found that this effect was consistent for leaf litter with varying N and lignin content and was not dependent on the mycorrhizal type of the neighboring trees. These findings support recent evidence that mycorrhizal fungi reduce litter decomposition rate only where low nutrient availability increases microbial demand for litter-derived C and nutrients. These results suggest that fine roots and their associated mycorrhizal hyphae stimulate litter decomposition in this ecosystem regardless of the functional group of mycorrhizal fungi, likely by supplying saprotrophic soil microbes with photosynthates.

Recent work linking biogeochemistry to mycorrhizal functional groups allows us to model these biogeochemical processes at the ecosystem scale, where tree species' mycorrhizal associations are generally known. Indeed, many have shown that dominant mycorrhizal associations can be used to predict ecosystem C and nutrient cycling dynamics (Phillips *et al.*, 2013; Soudzilovskaia *et al.*, 2015; Cheeke *et al.*, 2016; Rosling *et al.*, 2016; Frey, 2019) and response to environmental change (Midgley & Phillips, 2014; Mohan *et al.*, 2014; Terrer *et al.*, 2016; Liese *et al.*, 2018). Understanding when and where mycorrhizal fungi influence these processes will become increasingly important for predicting ecosystem responses to global change in light of expected shifts in tree species composition and relative mycorrhizal dominance of forests across the globe (Jo *et al.*, 2019).

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Author Contributions

AKL, FVJ, JHM, and MPA. designed the study; AKL, FVJ, and CRV established the study plots; CRV constructed the litterbags; AKL and CRV performed the extracellular enzyme assays; AKL and FVJ processed litter, soil, and root samples; MPA facilitated the PLFA assays; JHM facilitated soil moisture measurements; AKL and JHM performed the statistical analyses. AKL wrote the manuscript, and all authors contributed significantly to revisions. The authors declare no conflicts of interest.

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Supporting Information:

Fig. S1. Relationships between ectomycorrhizal (ECM)-associated tree basal area and a) the average percentage of fine root biomass of ECM-associated tree species in each plot (n=12), and b) the average percentage of biomarkers associated with arbuscular mycorrhizal (AM) fungi in each plot (n=12).

Fig. S2. (a) The effect of root exclusion treatment and treatment disturbance on volumetric soil moisture content in a subset of three plots shown over time during the study period. (b) The effect of monthly average soil moisture on litter decay rates in the subset of three continuously-monitored plots.

Table S1. Selected measurements of litter chemistry for representative samples of each species used in the study.

Table S2. Characteristics of the soil and fine roots within each study plot (n=12).

Table S3: Model results for the effect of litter species, soil trenching, and plot mycorrhizal dominance on the activities of enzymes on leaf litter.

Figure Captions:

Figure 1. Conceptual diagram of experimental design showing four leaf litter species incubated in three treatment conditions within plots that span a range of relative arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree basal area (n=12). Root color indicates treatment effects; dark brown, live roots in control treatments, gray, dead roots in trenched treatments, and both dead roots as well as new root growth in disturbed treatments.

Figure 2. Mean (± standard error) decomposition constant (*k*) for litter incubated in control, disturbed, and trenched subplots, shown overall (panel a; n=48) and individually by species (panel b; n=12). Data were pooled across the 12 study plots. Colors indicate subplot treatment. Letters refer to statistical differences between subplot treatments (Tukey's HSD; Table 1).

Figure 3. Decomposition rate (*k*) of each species of leaf litter within study plots (n=12) containing from 4 to 100% ectomycorrhizal (ECM)-associated tree basal area. Line and point color reflects subplot treatment. Note that axes vary as a result of species-level effects on *k*. Dashed lines indicate no effect of relative ECM tree basal area on litter decay rate.

Figure 4. Mass remaining (%) over time for litter of each species in each treatment with modeled decay functions for each treatment group. Points indicate the average mass of litter remaining in each subplot treatment at each time point, with values averaged across all plots (n=12). Bars indicate standard error of the mean. Lines are drawn from the negative exponential model fit to the values of mass remaining in litterbags at the three collection times. Line color indicates the treatment of the subplot where litter was collected.

Figure 5. Potential activities of selected enzymes on all four leaf litter species (pooled) in plots with increasing ectomycorrhizal (ECM) dominance, measured as percent tree basal area. Activities were measured after 608 days of litter decomposition. Color indicates subplot treatment. Solid lines indicate a significant interaction between subplot treatment and plot mycorrhizal dominance (β-glucosidase (BG): $F_{1, 79}$= 7.52, p= 0.0075; cellobiohydrolase (CBH): $F_{1, 79}$ = 3.98, p= .049; β-N-acetylglucosaminidase (NAG): $F_{1, 79}$= 3.93, p= 0.051; polyphenol oxidase (PPO): $F_{1, 78}$= 3.77, p= 0.056 for model interaction terms) and dashed lines indicate no interaction. Asterisk indicates an effect of subplot treatment on potential acid phosphatase (AP) activity ($F_{1,78}$= 6.22, p= 0.015).