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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Mycorrhizal roots slow the decay of belowground litters in a temperate hardwood forest

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Abstract

There is increasing evidence that plant roots and mycorrhizal fungi, whether living or dead, play a central role in soil carbon (C) cycling. Root–mycorrhizal–microbial interactions can both suppress and enhance litter decay, with the net result dependent upon belowground nutrient acquisition strategies and soil nutrient availability. We measured the net effect of living roots and mycorrhizal fungi on the decay of dead roots and fungal hyphae in a hardwood forest dominated by either sugar maple (*Acer saccharum*) or white oak (*Quercus alba*) trees. Root and fungal litter were allowed to decompose within root-ingrowth bags and root-exclusion cores. In conjunction with root effects on decay, we assessed foraging responses and root induced changes in soil moisture, nitrogen (N) availability and enzyme activity. After 1 year, maple root production increased, and mycorrhizal fungal colonization decreased in the presence of decaying litter. In addition, we found that actively foraging roots suppressed the decay of root litter (− 14%) more than fungal litter (− 3%), and suppression of root decay was stronger for oak (− 20%) than maple roots (− 8%). Suppressive effects of oak roots on decay were greatest when roots also reduced soil N availability, which corresponded with reductions in hydrolytic enzyme activity and enhanced oxidative enzyme activities. These findings further our understanding of context-dependent drivers of root–mycorrhizal–microbial interactions and demonstrate that such interactions can play an underappreciated role in soil organic matter accumulation and turnover in temperate forests.

Keywords Fungal necromass · Gadgil effect · Priming effect · Rhizosphere

Introduction

While there is increasing recognition that plant roots and their mycorrhizal fungal symbionts can actively modify decomposition processes (Frey 2019), our understanding of how interactions between roots, symbionts, and free-living microbes mediate soil carbon (C) balance remains limited. Roots and mycorrhizal fungi can suppress decay and slow C loss by intensifying competition for water and nutrients (Koide and Wu 2003; Fernandez and Kennedy 2016). The suppression of decay in the presence of mycorrhizal roots or enhanced decay following exclusion of mycorrhizal roots is termed 'the Gadgil effect' (Gadgil and Gadgil 1971, 1975). Conversely, roots and mycorrhizal fungi can enhance soil C decomposition and loss by releasing exudates to fuel microbial metabolism, known as a 'priming effect' or a 'rhizosphere priming effect' when microbial enzyme activity is stimulated in root adjacent soils (Kuzyakov et al. 2000). While most studies focus on the occurrence of either a Gadgil or rhizosphere priming effect, these context-dependent processes are not mutually exclusive (Fernandez and Kennedy 2016). To infer the net effect of mycorrhizal roots on decay processes we need a better understanding of which plant, soil, and litter characteristics promote the Gadgil effect over rhizosphere priming effects and vice versa. Moreover, most studies testing the Gadgil effect have focused on the effects of mycorrhizal roots on leaf litter decay and most studies testing priming effects have focused on soil organic matter (SOM) decay; thus, little is known about how these two processes affect the decay of root and fungal litters, despite the importance of root and

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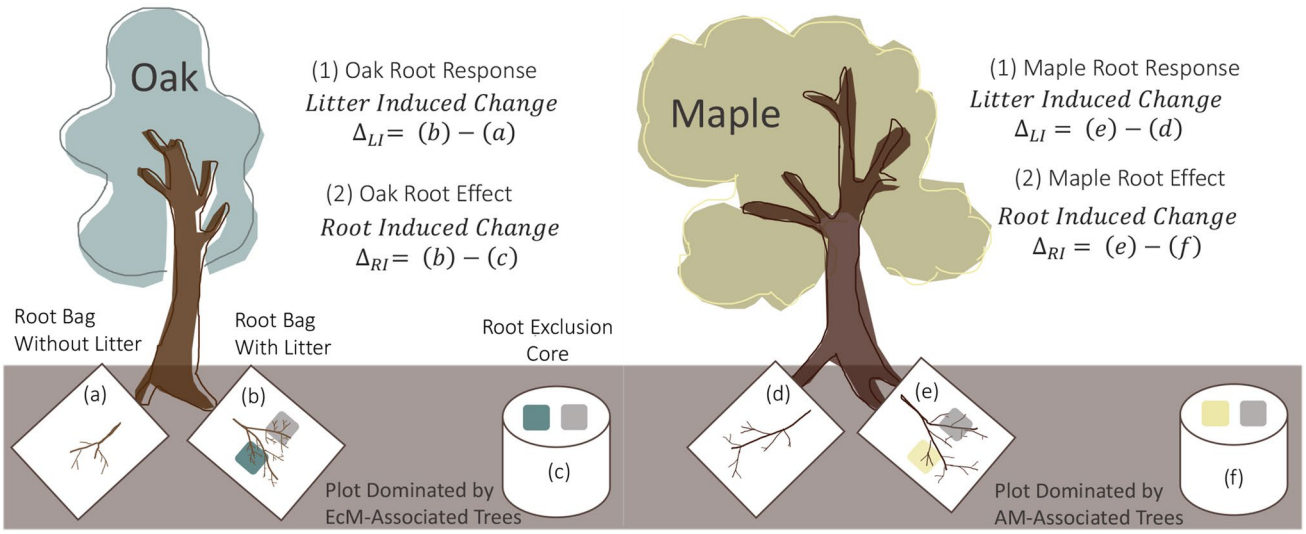
fungal-derived inputs to long-term soil C storage (Godbold et al. 2006; Clemmensen et al. 2013; Jackson et al. 2017). These knowledge gaps have hindered progress in incorporating root–microbe interactions into models (Moore et al. 2015) that can lead to improved predictions about the pace of climate change (Warren et al. 2015).

Tree species differ in a number of root traits related to belowground nutrient acquisition strategies, which in turn, may have differential effects on decomposition dynamics (Han et al. 2020). Belowground foraging strategies can range from 'do-it-yourself' (DIY) acquisition to 'outsourcing' acquisition to symbionts (Bergmann et al. 2020). Plants that use a DIY strategy prioritize efficient root construction by making thin roots with high specific root length (SRL). This may lead to greater competition for resources between roots and free-living soil microbes. Under these conditions, reduced rates of nutrient mineralization caused by less active soil microbes might be offset by fine root systems that can proliferate rapidly into nutrient patches (Hodge 2004; Liese et al. 2017). In contrast to a DIY strategy, roots might outsource resource acquisition to mycorrhizal symbionts, which result in greater resource competition between mycorrhizal fungi and soil saprotrophs. This leads to the prediction that heavily colonized roots or root systems with an increased number of mycorrhizal tips may be associated with (or lead to) a stronger Gadgil effect. As an alternative outsourcing strategy, plants may allocate C to root-associated microbial communities rather than selectively allocating C to mycorrhizal fungi and the same root traits that reflect a DIY strategy may also be associated with enhanced rates of root exudation (Sun et al. 2021). Thin absorptive roots with high SRL have been shown to enhance rhizosphere priming effects (Meier et al. 2017; Han et al. 2020). Given that interactions between plants and microorganisms are complex and can span the range from competitive to mutualistic, more studies are needed to determine if root traits and foraging strategies can be used to predict root–mycorrhizal–microbial interactions.

Mycorrhizal fungi are an extension of a plant's nutrient acquisition strategy, and the type of mycorrhizal fungi a plant associates with can influence the direction and magnitude of root effects on decay (Brzostek et al. 2015). The Gadgil effect is typically associated with ectomycorrhizal (EcM) fungi, as some species of EcM fungi produce oxidative enzymes to mobilize nitrogen (N) from organic matter (OM), deterring further decay by free-living saprotrophs (Bödeker et al. 2009; Shah et al. 2016; Zak et al. 2019). In contrast, arbuscular mycorrhizal (AM) fungi do not produce the oxidative enzymes required to degrade OM, though they may modify the activity of free-living saprotrophs via water and nutrient reduction or through hyphal turnover and exudation (Bunn et al. 2019). In general, EcM-associated plants are believed to induce greater rhizosphere priming effects in forests (Phillips and Fahey 2006; Sulman et al. 2017), though mesocosm studies indicate that rhizosphere priming by AM-associated plants may be equal to Chen et al. (2018) or greater than (Wurzburger and Brookshire 2017) EcM-associated plants. Furthermore, it is unclear if the Gadgil effect is specific to EcM-associated gymnosperms or if it also occurs in forests containing deciduous angiosperm trees (Netherway et al. 2021). Given the potential for mycorrhizal types to differ in their interactions with soil microbes (Cheeke et al. 2017) more studies need to be conducted in temperate hardwood forests containing both EcM- and AM-associated tree species.

Soil nutrient availability is a key modulator of both Gadgil and priming effects. Much of the support for the Gadgil effect comes from studies conducted in coniferous forests, where soil nitrogen (N) is limited and held in slow-decaying organic forms (but see Lin et al. 2019 and Lang et al. 2020). However, paradoxically, exudation rates are often greatest in low N soils (Pausch and Kuzyakov 2018), as increased exudation can stimulate microbial N transformations and turnover (Dijkstra et al. 2013; Cheng et al. 2016; Meier et al. 2017; Yin et al. 2018). Whether mycorrhizal roots inhibit or facilitate other microbes in low nutrient soils likely depends on the nutrient content or quality of litter inputs themselves (Smith and Wan 2019). It is thought that higher quality litter inputs (lower C:N ratios), in the earlier stages of decay increase carbon use efficiency among free-living decomposers and can limit the advantage of EcM fungi in acquiring organic N, weakening the Gadgil effect (Fernandez et al. 2020). More studies are needed to disentangle the effects of soil and litter nutrient conditions on mycorrhizal interactions with saprotrophs.

We sought to evaluate the net effect of living roots and mycorrhizal fungi on decaying roots and fungi differing in their resource quality within forest stands differing in mycorrhizal type and soil nutrient conditions. We pose the following questions (i) Do root traits related to foraging (i.e., root production, morphology, and mycorrhizal colonization) reflect root involvement in decomposition? (ii) How do living roots and mycorrhizal fungi influence the decay of dead roots and fungi in a hardwood forest for both an EcM (white oak) and AM-associated (sugar maple) tree species? We paired root ingrowth bags with root exclusion cores (See Fig. 1) to measure root foraging responses and effects on decomposition (mass loss). In addition to root and fungal mass loss, we measured how roots altered the availability of limiting resources (e.g., water and nitrogen), N cycling rates and rhizosphere enzyme activity to test for the occurrence of a rhizosphere priming effect. We hypothesize that (i) trees respond to litter additions by altering belowground foraging behavior (by either increasing root proliferation or mycorrhizal colonization) and (ii) the presence of mycorrhizal roots influences mass loss from root and fungal litter.



Litter type	Initial C (%)	Initial N (%)	Initial C:N
Fungal Litter	44.52 ± 0.53[a]	5.55 ± 0.67[a]	8.28 ± 1.04[a]
Oak Root Litter	50.70 ± 0.98[b]	1.25 ± 0.04[b]	40.75 ± 0.74[b]
Maple Root litter	48.03 ± 0.41[c]	1.30 ± 0. 02[b]	36.95 ± 2.03[b]

Fig. 1 Schematic of experimental design and table of litter carbon (C) and nitrogen (N) values. Litter induced changes in root foraging behavior (morphology and growth) were assessed after 92 and 365 days of root ingrowth. Root (and mycorrhizal fungal) induced changes in litter mass loss were determined after 92 and 365 days of decay for *Quercus alba* (Oak), *Acer saccharum* (Maple) and *Melinomyces bicolor* (fungal) litter. For each litter type C and N values are presented as mean values ± SE ($n = 3$). For a particular C or N value, means not sharing a letter are significantly different ($P < 0.05$)

Further, we hypothesize that the magnitude of root effects on decay differs between tree species and litter types. By measuring both root effects and responses to decay we hoped to enhance our knowledge of how belowground acquisition strategies might contribute to soil C and nutrient cycling in forest ecosystems.

Materials and methods

Site description

We conducted this work at Moores Creek, a mature hardwood forest located in south-central Indiana (39°05′ N, 86°28′ W; MAP = 1200 mm; MAT = 11.6 °C) in 20 × 20 m forest plots previously established by Midgley et al. (2015). Midgley et al. (2015) selected forest stands in which AM-associated tree species or EcM-associated trees species comprised greater than 85% of stand basal area. AM plots include a mixture of the following species: *Acer saccharum*, *Liriodendron tulipifera, Prunus serotina* and *Sassafras albidum*. EcM plots contain a mixture of *Carya glabra*, *Fagus grandifolia*, *Quercus alba* and *Quercus rubra*. We chose three plots in which *Acer saccharum* (hereafter referred to as "maple") was the predominant AM-associated canopy species and another three plots in which *Quercus alba* (hereafter referred to as "oak") was the predominant EcM-associated canopy species. We chose these two species because they are dominant species across much of the Northeast and Midwest (Jo et al. 2019) and have been shown to have differential effects on decay (Brzostek et al. 2015; Malik 2019). Within each plot, we selected four trees (12 trees total per species). Paired oak and maple plots were organized in three forest blocks according to location (Fig. S1).

Decomposition measurements

We collected root litter in September of 2017, for additional details see SI methods. We chose to decay oak root litter in the presence of oak roots and maple root litter in the presence of maple roots in order to capture the decay environment that occurs most commonly for these litters (Ayres et al. 2009). This allowed us to test for the presence of either Gadgil or priming effects in 'home soils' for both oak and maple trees. To generate fungal litter, we grew *Melinomyces bicolor* hyphal plugs in 50 ml of half strength potato dextrose broth. Flasks containing *M. bicolor* cultures were kept in the dark at 20 °C on an orbital shaker at 80 rpm. After 30 days, we thoroughly rinsed *M. bicolor* hyphae with DI

water and dried at 25 °C for 48 h (Fernandez and Kennedy 2018).

Despite not being a site-specific EcM fungal species, we chose *M. bicolor* because it is common to temperate and boreal forest soils (Grelet et al. 2009) and decays on a time scale more similar to plant litter, with fungal mass remaining after three months of decay (Fernandez and Kennedy 2018). *M bicolor* necromass has also been utilized in a previous study conducted at this site assessing decay dynamics and fungal necromass decomposer communities in both AM and EcM dominated tree stands (Beidler et al. 2020). The generation of AM fungal necromass in the quantity required for this study was methodologically infeasible; thus, we chose to deploy a common fungal litter to compare the magnitude of oak and maple root effects on fungal decay as well as to compare fungal decay results to previous studies (Fernandez and Kennedy 2018; Beidler et al. 2020).

To determine initial litter C:N values, we ground dried root and fungal material to a powder using a GenoGrinder (SPEX® SamplePrep) and analyzed the ground material for total C and N (Elemental Combustion System 4010; Costech Analytical Technologies, Valencia, CA, USA). We constructed litter bags (4.5 cm × 4.5 cm) from 0.2 mm nylon mesh (4.5 cm × 4.5 cm) and filled them with either 100 mg of root litter for each tree species or 25 mg of *M. bicolor* fungal hyphae. We determined that these masses were large enough to detect significant changes in mass loss (Träger et al. 2017; Beidler et al. 2020) and could serve as realistic proxies for organic nutrient patches within the soil environment (Hodge 2004).

We incubated root and fungal litters in two environments—inside root ingrowth bags and root exclusions (details below; Fig. 1). We constructed root ingrowth bags (20 cm × 25 cm) from polyester fabric with a 0.5 mm mesh size (Eissenstat et al. 2015). During the first week of May 2018, we tracked a live woody root (> 3 mm in diameter, ~ 25 cm in length) to a focal tree (at a depth of 10–15 cm) and carefully removed all lateral absorptive roots. We pruned roots to initiate new absorptive root growth and to measure root foraging responses to litter decay. For each tree, we placed root and fungal litter in half of the root bags, on either side of a pruned root (Fig. 1). We then filled root bags with root free soil or soil picked free of roots and passed through a 2 mm sieve (~ 300 g) collected from around each tree (10–15 cm depth). We collected a subsample of soil to determine initial soil pH and total soil C and N for each focal tree (Fig. S1). We installed two sets of root bags (one bag with litter and one bag without litter) to allow for two separate harvests (3 months after installation and 1 year after installation). In total we installed 48 root bags per tree species (4 root bags × 4 trees × 3 plots).

We made root exclusions by driving PVC pipe (15.2 cm diameter) to a 30 cm depth. We then removed the PVC pipe (keeping the soil column inside intact) and wrapped the bottom with 1 µm mesh to exclude roots and mycorrhizal hyphae. We left exclusion cores to equilibrate for a year ($n = 6$, 1 per plot). At the same time, we buried litter bags at a similar depth inside of exclusions. We collected litter bags in August of 2018 (after 3 months) and May of 2019 (after 1 year). Following collection, litter bags were brushed free of soil and any living roots that had colonized the outside of litter bag were carefully removed with forceps. To ensure that the mass observed in litter bags was from decaying root litter and not from the in-growth of newly produced roots, the contents of each litter bag was observed under magnification (2.25 ×) and any living roots (determined by differences in root coloration and turgidity) were removed with forceps. Living roots were brighter in color and were turgid or not easily broken when pulled with forceps (Vogt and Persson 1991). However, when present, living roots were largely confined to the outer mesh of the litter bag. Following processing, litter bags were oven-dried at 60 °C for 48 h and massed.

To test for the potential disturbance effects of soil sieving and an additional layer of mesh fabric on mass loss from litterbags within root bags vs. exclusions, we conducted a follow-up decomposition experiment where we decayed litter inside (enclosed litter bag) and outside of root bags (non-enclosed litter bag) within the same PVC exclusions, the following summer. We found no differences in mass loss for litter decaying inside of the enclosed and non-enclosed litter bags ($p = 0.560$, Table S1), indicating that bag environment did not significantly affect mass loss.

Root trait measurements

We harvested root bags by block in the same order that we installed them. During processing we excavated and carefully cleaned the intact root networks within root bags. We excluded any root bags containing dead roots from subsequent analyses (see Table S2 for information on pruning recovery). Following cleaning, we removed absorptive roots (orders 1–2) from transportive roots (3rd order and higher) using stream-order classification (Guo et al. 2008). We floated roots in a transparent tray and imaged them using an Epson Expression 100000XL scanner (300 dpi). We analyzed images for root length, average diameter, root volume, and tip number using Win-RHIZO (Regent Instruments Inc 2009). We then dried and weighed roots (60 °C for 48 h) to determine root tissue density (g cm^{-3}) and specific root length (m g^{-1}).

Soil measurements

To minimize disturbance of soil inside of exclusions, we took point measurements of soil volumetric water content

(VWC) using a Hydrosense II soil–water sensor (Campbell Scientific). At the end of the study (365 days), when root bags were fully colonized by living roots and pruning related disturbances had likely subsided, we sampled soils from root bags and exclusions for differences in nitrogen (N) availability and enzyme activities using the methods of Brzostek et al. (2015) and Midgley and Phillips (2019). We extracted soil inorganic N (NH4 + –N and NO3–N; μg g^{-1}) from a 5 g soil subsample using 10 mL of a 2 M KCl solution. We determined N mineralization and nitrification rates (μg g^{-1} day^{-1}) using an additional set of soil subsamples which we incubated for two weeks at 25 °C, subsequently extracted with 10 mL of 2 M KCl solution and analyzed on a Lachat QuikChem 800 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA).

We performed assays for the following enzymes: b-1,4-*N*-acetylglucosaminidase (NAGase- involved in N degradation), β-Glucosidase (BG; involved in degradation of labile C), oxidative (OX) enzyme activity (the sum of peroxidase and phenol oxidase activities; enzymes involved in the degradation of complex C). We measured potential enzyme activities for soils within root bags and PVC exclusion cores, as well as for rhizosphere soils in root bags with and without litter additions. We performed both colorimetric (OX) and fluorometric (BG and NAG) assays by preparing soil slurries (1.5 g soil and 100 mL of sodium acetate buffer per sample: pH 5) and adding them to microplates in triplicate. To determine BG and NAG activities, we added methylumbelliferone (MUB) substrate, incubated the microplates in the dark at 23 °C for either two (NAG) or five (BG) hours and read the plates using a microplate fluorometer (365 nm excitation and 450 nm emission). To determine OX enzyme activities, we added L-3,4-dihydroxyphenylalanine (L-DOPA) substrate, incubated microplates in the dark at 23 °C for four hours and read the plates using a microplate spectrophotometer (absorbance at 460 nm). We corrected potential enzyme activities (μmol g^{-1} h^{-1}) for controls, quenching, and dry soil weights (Midgley and Phillips 2019).

Calculations and statistical tests

We calculated root mass loss by subtracting ash-free dry mass of the remaining litter from the ash-free dry mass of the initial input for each litter bag. We calculated pruning recovery as the percentage of woody roots that grew new absorptive roots following pruning (Eissenstat et al. 2015). We calculated specific root length[1+2] (SRL), as the length of the 1st + 2nd order roots divided by the corresponding 1st + 2nd order root dry weight. We calculated root tissue density[1+2] (RTD) as 1st + 2nd order root dry weight divided by 1st–2nd order root volume. We calculated root branching intensity as the number of tips per 1st + 2nd root length (Liese et al. 2017). We determined total root length by combining measurements for the absorptive and transportive roots and dividing by the duration of the growth period to determine new root length production (cm day^{-1}) following pruning. We determined the percentage mycorrhizal colonization for oak roots by dividing the number of first-order roots colonized by EcM fungi by the total number of first order roots examined; to determine AM colonization intensity for maple roots we used the grid line intersect method (Brundrett et al. 1996). Five maple root samples were damaged during clearing and omitted from mycorrhizal colonization calculations.

We determined the magnitude of root effects for each plot by subtracting measurements taken from exclusions from root bag treatments to calculate root induced changes in mass loss, soil moisture content, N cycling and enzyme actives (Fig. 1). We determined the magnitude of litter effects on root foraging for a given tree by subtracting measurements taken from root bags without litter additions from root bags with litter additions to calculate the litter induced changes in root foraging traits (Fig. 1). We performed one sample T tests to determine if mean changes differed from zero.

Because mass remaining, and VWC data are bounded by zero and one, they often did not meet the assumptions of normality and/or homoscedasticity. As an alternative to transformation, we fit generalized linear mixed-effect (GLME) models based on a beta distribution with a logit link function using the glmmTMB package in R (Douma and Weedon 2019). Fixed effects included root treatment, tree species, incubation time and forest block. Plot nested within block was included as a random effect. We included soil VWC to control for potential moisture differences between treatments and plots. We also included treatment, species, and time interactions. We performed type III Wald chi-square tests to determine statistical significance of GLME models ($\alpha = 0.05$; Anova.glmmTMB function).

We assessed the effect of root treatment, tree species and potential interactions on soil variables using linear mixed-effect (LME) models with plot nested in block as a random factor. We also used LME models to test the fixed effects of litter addition, tree species, growth period and all interactions on root foraging traits. We included tree nested in plot as a random effect. We used the ANOVA function in the car package to generate analysis of deviance tables for LME models (Type III Wald Chi-squared tests; $\alpha = 0.05$; Fox and Weisberg 2019; See SI for ANOVA tables). We explored interactions using Tukey post hoc tests in the lsmeans package (Lenth 2018). We performed regression analysis to test for significant relationships between correlated continuous variables ($\alpha = 0.05$). We carried out all statistics using R 4.0.3 (R Core Team 2020).

Results

Root responses to presence of decaying litter

We found that morphological root traits did not differ between tree species, except for average diameter$_{1+2}$ and tissue density$_{1+2}$ (Table 1). First and second order oak roots were thinner and denser on average. New root production was higher for oak roots during the first three months (oak, 2.5 ± 0.3, maple, 1.3 ± 0.2 cm day^{-1}; $t = -1.6$, $p < 0.001$) but did not differ between species after a year ($t = -0.11$, $p = 0.9$; Table S3). Overall, recovery from pruning or the percentage of roots that grew new absorptive root length, was greater for roots grown in soil with litter additions (79%) when compared to soils without litter additions (60%; $\chi^2 = 4.3$, $p = 0.04$; Table S2). On average root trait values did not differ between soil treatments for either sampling date (Table 1).

Significant litter induced changes in root production and mycorrhizal colonization were detected for maple roots after 365 days of growth (Fig. 2). After 1 year, maple root production was 70% higher and mycorrhizal colonization was 8% lower in the presence of decaying litter (Table S4). After one year of root ingrowth, rhizosphere enzyme activities were similar between tree species and soil treatments, except for NAGase, which was twice as high in oak rhizosphere soils in the presence of decaying litter (Table 2). Moreover, for oak roots growing in soils with litter additions, rhizosphere activity of C-degrading enzymes related negatively to mycorrhizal colonization (slope: − 0.022; $R^2 = 0.58$; $p = 0.004$) and positively to root production (slope: 0.703; $R^2 = 0.44$; $p = 0.02$) (see Fig. 3).

Table 1 Average root foraging trait values ± SE *Quercus alba* (Oak) and *Acer saccharum* (Maple) roots growing in soils with and without litter additions

	Avg. Diameter$_{1st+2nd}$ (mm)		SRL$_{1st+2nd}$ (m g^{-1})		Root tissue density (g cm^{-3})		Branching intensity (no. cm^{-1})	
Growth period (days)	92	365	92	365	92	365	92	365
Oak roots								
With litter	0.466 ± 0.05^a	0.462 ± 0.02^a	32.6 ± 3.9^a	33.4 ± 3.9^a	0.173 ± 0.01^a	0.217 ± 0.02^a	1.98 ± 0.09^a	2.37 ± 0.09^a
Without litter	0.480 ± 0.06^a	0.434 ± 0.02^a	31.1 ± 4.0^a	32.8 ± 4.0^a	0.186 ± 0.01^a	0.184 ± 0.05^a	1.94 ± 0.18^a	2.45 ± 0.15^a
Maple roots								
With litter	0.605 ± 0.08^b	0.567 ± 0.01^b	25.9 ± 1.8^a	31.7 ± 2.6^a	0.141 ± 0.01^b	0.129 ± 0.01^b	2.13 ± 0.13^a	2.07 ± 0.14^a
Without litter	0.610 ± 0.08^b	0.574 ± 0.03^b	28.1 ± 4.5^a	28.1 ± 4.5^a	0.156 ± 0.03^b	0.159 ± 0.02^b	1.96 ± 0.13^a	2.32 ± 0.14^a

For a particular trait, means not sharing a letter are significantly different ($P < 0.05$)

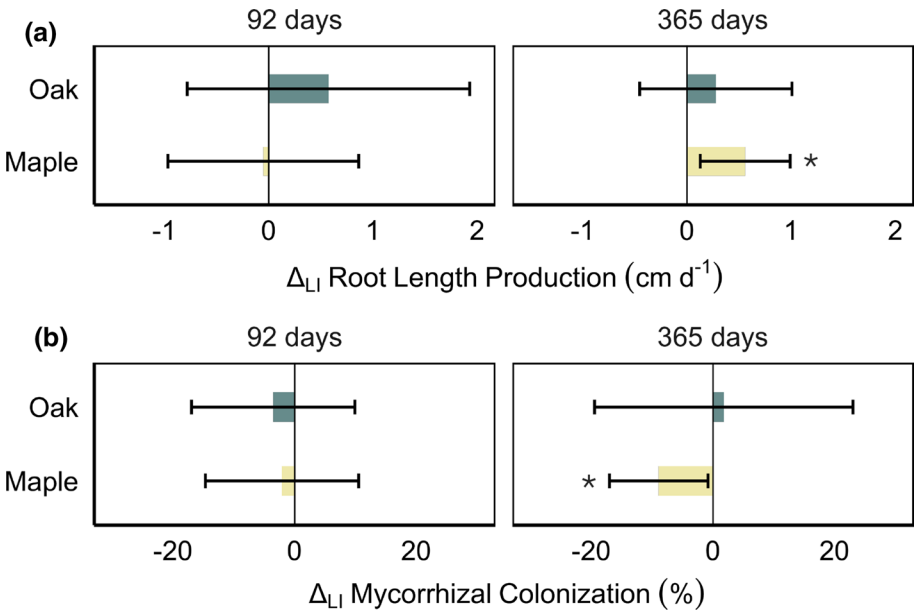


Fig. 2 Litter-induced changes (Δ_{LI}) in *Quercus alba* (Oak) and *Acer saccharum* (Maple) **a** root length production and **b** mycorrhizal colonization rates at 3 months (92 days) and 1 year (365 days). Error bars represent 95% confidence intervals (CIs). One sample T-tests were performed to determine whether root induced changes differed significantly from zero (denoted by an asterisk*; see Table S5 for *T*-test results)

Table 2 Average rhizosphere β-Glucosidase (BG), b-1,4-*N*-acetylglucosaminidase (NAG), and oxidative (OX) enzyme activities ± SE for *Quercus alba* (Oak) and *Acer saccharum* (Maple) roots growing in soils with and without litter additions after 365 days

Rhizosphere enzyme activity 365 days	*n*	OX (μmol g^{-1} h^{-1})	BG (μmol g^{-1} h^{-1})	NAG (μmol g^{-1} h^{-1})
Oak roots				
With litter	11	1.01 ± 0 12[a]	0.889 ± 0.14[a]	0.280 ± 0.04[a]
Without litter	9	1.11 ± 0.07[a]	0.932 ± 0.19[a]	0.241 ± 0.03[ab]
Maple roots				
With litter	7	0.906 ± 0.06[a]	0.863 ± 0.04[a]	0.129 ± 0.02[c]
Without litter	11	0.980 ± 0.08[a]	0.829 ± 0.06[a]	0.150 ± 0.02[bc]

For a particular enzyme, means not sharing a letter are significantly different ($P < 0.05$: Table S6)

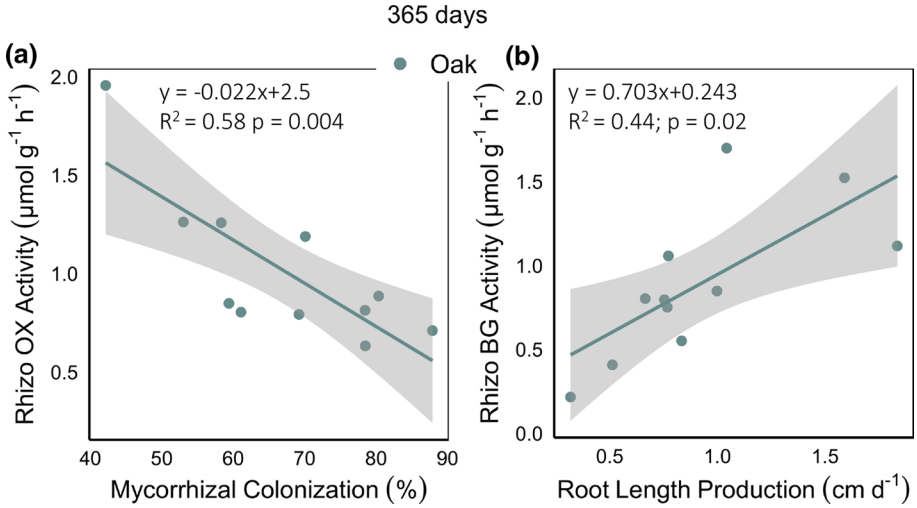


Fig. 3 Relationships between *Quercus alba* (Oak) root traits and C-degrading enzyme activity in rhizosphere or root adjacent soils. **a** Relationship between oak root mycorrhizal colonization and rhizosphere oxidative (OX) enzyme activity **b** relationship between oak root length production and rhizosphere β-Glucosidase (BG) activity

Root effects on decay

We found that the presence of foraging roots suppressed decay and that the magnitude of root reductions in litter mass loss depended on tree species, time period, and litter type (Fig. 4a). The suppression of decay was greater for oak root litter. When averaged across time periods, mass loss of oak root litter decreased by 20% and maple root litter decreased by 8% in the presence of living roots. For both oak and maple trees, root effects on decay decreased over time (Fig. 4b), supported by a significant treatment by time interaction with respect to root mass remaining ($\chi^2 = 7.2$, $p = 0.007$; Table S8). When averaged across species, root mass remaining in exclusions after three months (62.1 ± 2.9%) was similar to root mass remaining in the presence of living roots after one year (67.9 ± 1.8%; $t = -1.9$; $p = 0.2$). Relative to root litter, fungal litter decomposed rapidly, with 85% of mass loss occurring in the first 92 days, more than triple that of root litter (~25% mass loss after 92 days; Fig. 4b). However, the only significant root induced change in fungal decay was a 6% decrease in fungal mass after 365 days in the presence of maple roots ($t = -3.02$; $p = 0.02$; Table S8).

Exclusion of roots and mycorrhizae also altered soil conditions, with the direction of root induced changes differing between tree species. Across time periods the presence of oak roots increased soil VWC (+2.1 ± 0.4%; $t = 4.78$, $p < 0.001$) while the presence of maple roots decreased soil VWC (− 4.0 ± 1.1%, $t = -3.4$, $p = 0.004$; Table S8). After 1 year, the presence of roots altered soil N availability without altering soil pH (Table 3). Oak roots decreased soil inorganic N (− 4.7 ± 6.9 μg g^{-1}; $t = -2.26$, $p = 0.05$) while maple roots increased soil inorganic N (+9.12 ± 4.5 μg g^{-1}; $t = 5.39$, $p = 0.002$; Table S9). Differences in soil inorganic N concentrations were likely due to differences in N cycling rates in the presence of roots (Table 3). Nitrification rates were 5.5 × lower and N mineralization rates were 2.5 × higher when oak roots were present. Whereas, nitrification rates were 4 × higher and N mineralization rates were 7 × higher when maple roots were present. However, it is important to note that when roots were excluded, average soil inorganic N concentrations were similar between maple and oak plots ($t = 0.89$, $p = 0.80$; Table 3).

We also detected significant oak root induced changes in carbon (C) degrading enzyme activities (Fig. 5). On average oak roots reduced β-Glucosidase (BG) activities

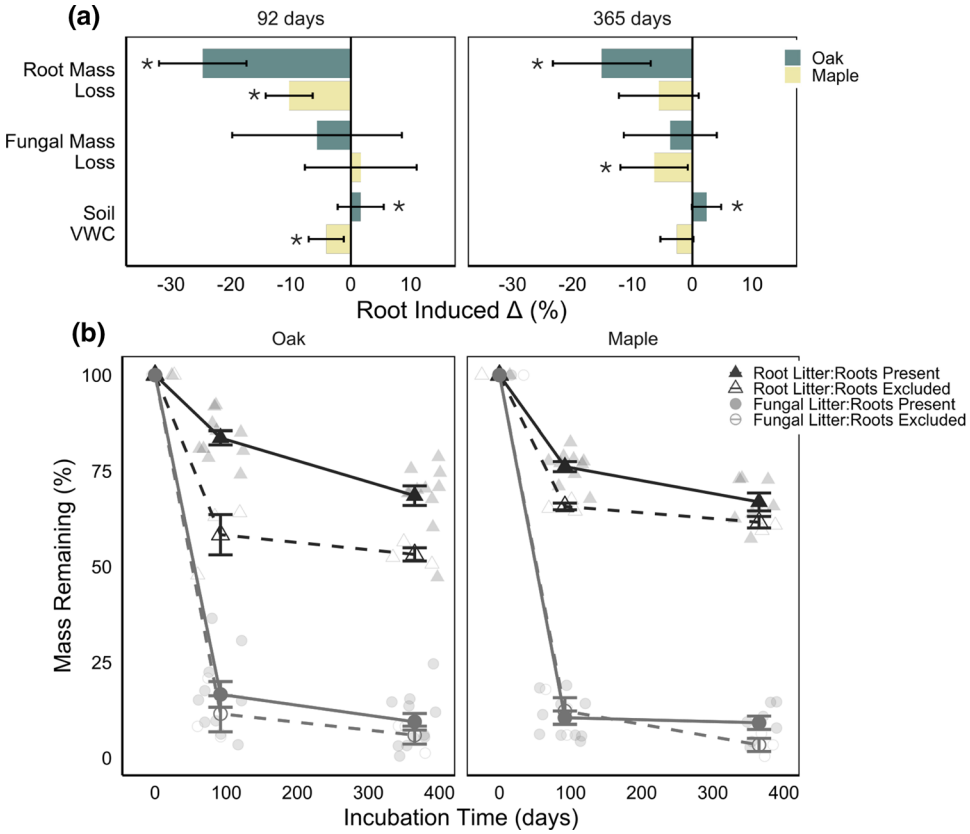


Fig. 4 **a** Root (and mycorrhizal fungal) induced changes in *Quercus alba* (Oak) and *Acer saccharum* (Maple) percent root litter mass loss (Root Mass Loss) and *Melinomyces bicolor* necromass loss (Fungal Mass Loss), as well as soil volumetric water content (Soil VWC) at 3 months (92 days) and 1 year (365 days). Error bars represent 95% confidence intervals (CIs). One sample *T*-tests were performed to determine whether root induced changes differed significantly from zero (denoted by an asterisk*: see Table S9 for *T*-test results) **b** Average mass remaining ± SE of root and fungal litter through time

Table 3 Average soil nitrogen (N) cycling metrics (total inorganic N, Nitrification and N mineralization rates) ± SE for soils surrounding decaying litter in the presence and absence of *Quercus alba* (Oak) and *Acer saccharum* (Maple) roots after 365 days

	n	pH	Total inorganic N ($\mu g\ g^{-1}$)	Nitrification rate ($\mu g\ g^{-1}\ day^{-1}$)	N Min. rate ($\mu g\ g^{-1}\ day^{-1}$)
Oak					
Roots present	11	3.93 ± 0.06^{a}	8.22 ± 1.6^{a}	0.02 ± 0.005^{a}	3.46 ± 0.52^{a}
Roots excluded	3	3.84 ± 0.06^{a}	12.7 ± 3.5^{bc}	0.11 ± 0.05^{ab}	1.29 ± 0.87^{bc}
Maple					
Roots present	7	5.25 ± 0.20^{b}	24.5 ± 2.1^{ab}	3.27 ± 0.13^{c}	2.63 ± 0.14^{ab}
Roots excluded	3	5.02 ± 0.25^{b}	16.0 ± 2.7^{c}	0.76 ± 0.70^{b}	0.38 ± 0.54^{c}

For a particular measurement, means not sharing a letter are significantly different ($P < 0.05$: Table S10)

by ~ 70% (roots present: 0.494 ± 0.07 μmol g^{-1} h^{-1}; roots absent: 1.05 ± 0.17 μmol g^{-1} h^{-1}; $\chi^2 = 32.7$, $p < 0.001$) and enhanced OX enzyme activities by ~ 50% (roots present: 0.912 ± 0.09 μmol g^{-1} h^{-1}; roots absent: 0.543 ± 0.03 μmol g^{-1} h^{-1}; $\chi^2 = 9.57$, $p < 0.001$; Table S10). Root induced changes in mass loss were not directly related to root induced changes in soil conditions within oak or maple plots. However, we found that oak root induced changes in soil inorganic N concentrations were positively related to root induced changes in BG activity (slope: 0.01; $R^2 = 0.30$; $p = 0.05$) and negatively related to OX enzyme activities (slope: − 0.026; $R^2 = 0.26$; $p = 0.06$). Though these relationships were only moderately significant, in soils where oak roots reduced N availability, BG activity tended to be lower and OX enzyme activities tended to be higher in the presence of roots and mycorrhizal fungi (Fig. 6).

Discussion

Approximately 50–70% of soil C is thought to originate from belowground inputs (Godbold et al. 2006; Clemmensen et al. 2013), yet the degree to which roots, and mycorrhizal fungi influence soil C storage is poorly understood in temperate forests. We hypothesized that trees would respond to root and fungal litter additions by altering root morphology or

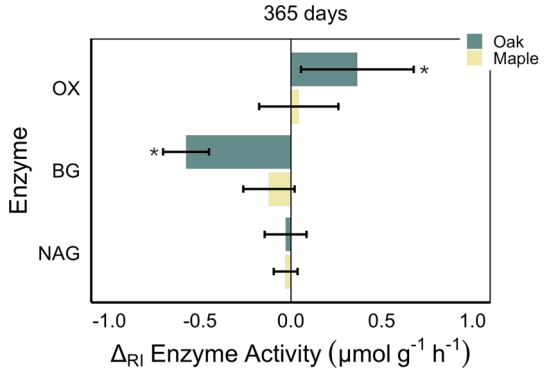


Fig. 5 Root (and mycorrhizal fungal) induced changes in soil organic matter (SOM) degrading extracellular enzyme activities in soils dominated by either oak (*Quercus alba*) or maple (*Acer saccharum*) roots on the final sampling date (365 days). Data is presented for the following enzymes: β-Glucosidase (BG), b-1,4-N-acetylglucosaminidase (NAG), and oxidative (OX) enzymes (phenol oxidase and peroxidase activities summed). Error bars represent 95% confidence intervals (CIs). One sample T-tests were performed to determine whether root induced changes differed significantly from zero (denoted by an asterisk*; see Table S12 for T-test results)

increasing mycorrhizal colonization, and that the magnitude of root effects on decay would differ between tree species and litter types. We found that maple roots grew faster and were less colonized by AM fungi in the presence of decaying litter (Fig. 2). Oak roots with increased rates of EcM colonization were associated with decreased rhizosphere oxidative enzyme (OX) activity in the presence of decaying litter (Fig. 3). However, root foraging traits and rhizosphere enzyme activities did not relate directly to root induced changes in decay. We predicted that suppressive effects of mycorrhizal roots on decay would be greater for lower quality litters (roots) than higher quality litters (fungi), and greater for oak roots (which associate ECM fungi) than maple roots (which associate with AM fungi). We found that living mycorrhizal roots inhibited the decay of root litter to a greater degree than fungal litter (Fig. 3), and the magnitude of this effect was greater for oak roots growing in N limited soils which also tended to have reduced BG activity and enhanced activity of oxidative (OX) enzymes (Fig. 6). Collectively, our results show that mycorrhizal roots can play a key role in the carbon and nutrient dynamics of belowground litter and the potential for litter quality and tree species identity to modify that role.

Root responses to presence of decaying litter

Maple and oak roots differed in their foraging response to the presence of litter. Maple root production increased, and mycorrhizal colonization decreased (Fig. 2), consistent with other root pruning studies (Eissenstat et al. 2015). Maple roots are believed to forage more by proliferating roots rather than by allocating resources to mycorrhizal fungi, reflecting a 'DIY' strategy (Bergmann et al. 2020). If greater root proliferation decreased nutrient or water availability for free-living microbes, this might explain the slight suppression of litter decay by maple roots. Oak root foraging traits did not respond to litter additions (Table 1) and this lack of root response might be indicative of greater reliance on mycorrhizal foraging away from roots (Chen et al. 2018; Cheng et al. 2016). It is thought that EcM fungal species producing cords or rhizomorphs that span larger distances from root tips have greater enzymatic capabilities and thus might contribute to a stronger Gadgil effect in hyphosphere (soils surrounding hyphae) rather than rhizosphere soils (Tedersoo and Smith 2013). Furthermore, Oak root mycorrhization was negatively related to oxidative enzyme activity in rhizosphere soils, indicating that mycorrhizal fungi were likely not enhancing OX activities in root adjacent soils (Fig. 3). Instead, saprotrophic fungi (Baldrian 2008) or even bacteria (Lladó et al. 2017) might have been the primary producers

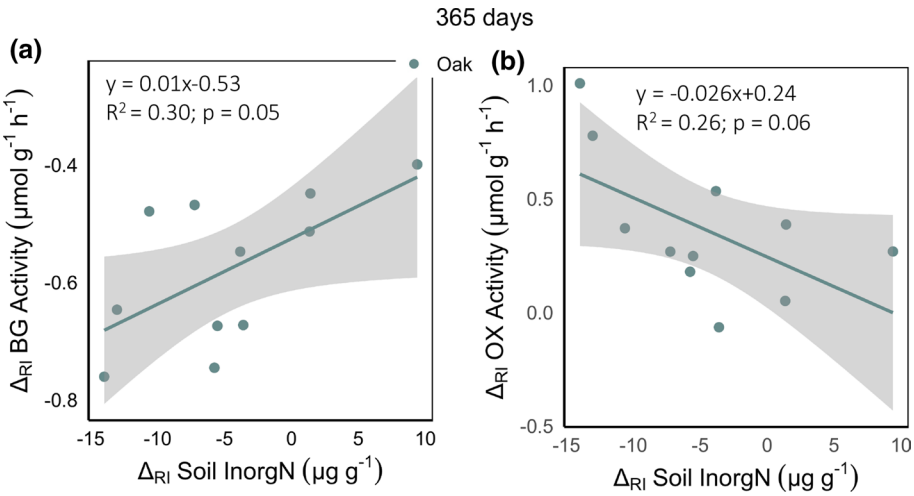


Fig. 6 Relationships between *Quercus alba* (Oak) root induced changes (Δ_{RI}) in total soil inorganic nitrogen (N) and **a** β-Glucosidase (BG) and **b** oxidative (OX) enzyme activities after 365 days. The shaded confidence region around the regression line is a pointwise 95% confidence interval. For root induced change calculations refer to Fig. 1

OX enzymes in rhizosphere soils. Future studies should relate enzyme production to fungal community composition at increasing distances from the root to better understand the competitive dynamics between mycorrhizal fungi and free-living soil saprotrophs.

Root effects on decay

Our results suggest that mycorrhizal roots can indirectly influence decay processes by modifying the soil environment surrounding decomposing litter. Ingrowth of maple roots reduced soil moisture (Fig. 4a)) which might have contributed to the suppression of maple root decay (Koide and Wu 2003). Surprisingly, the presence of oak roots increased the moisture content of surrounding soils. Oak roots and associated EcM mycelial networks may have enhanced soil water retention by promoting soil aggregate formation and stability (Querejeta et al. 2012; Querejeta 2017). Oak and maple roots both increased N mineralization rates; however, maple roots increased nitrification rates; whereas, oak roots decreased nitrification rates (Table 3). These results are consistent with previous studies reporting elevated nitrification in maple-dominated stands compared to adjacent oak-dominated stands (Vitousek et al. 1982; Lovett and Mitchell, 2004). The reasons for the differences are unclear but may relate root-promotion of distinct microbial communities. In a study conducted on the same site, Mushinski et al, (2021) reported that plots dominated by AM trees (many of which were maple-dominated) had soil microbial communities with fourfold more N cycling genes than ECM-dominated plots (many of which were oak dominated). It also suggests that maple roots may have been less limited by N and instead could have been competing for water or phosphorus (DeForest and Snell 2020).

Given that oak roots stimulated N cycling to a lesser degree than maple roots, N availability may have contributed to a stronger Gadgil effect. Root-induced changes in N cycling in oak plots corresponded with reductions in the activity of β-glucosidase (Fig. 6), suggesting that the cost of synthesizing N-rich enzymes may have slowed saprotrophic activity. Mycorrhizal hyphae, especially those produced by EcM fungi, extend beyond the rhizosphere into the surrounding soil, and mycorrhizal hyphae independent of roots have been shown to reduce BG activity and slow root litter decay (Lin et al. 2019). At the same time, EcM fungi can accelerate N cycling via priming effects (Meier et al. 2015). Thus, the accelerated rates of N mineralization in the oak soils (Table 3) may not have provided sufficient N to satisfy the N demands of mycorrhizal hyphae in the soil surrounding decaying litter. Thus, to better understand root vs. mycorrhizal contributions to soil carbon and nutrient cycling, future studies should compare microbial parameters (microbial community composition, enzyme activities and respiration rates) within rhizosphere and hyphosphere soils under a range of soil conditions.

Previous studies have shown that the presence of white oak and sugar maple roots had contrasting effects on the decay of species-specific leaf litter (Brzostek et al. 2015) and no effect on wood decay (Malik, 2019).

Moreover, a recent study testing the Gadgil effect in a northern hardwood forest found that the presence of both EcM and AM associated roots stimulated leaf litter decay (Lang et al. 2020). Inconsistencies among studies testing the Gadgil effect in temperate forests may be attributed to variation in the quality of litter inputs (Fernandez and Kennedy 2016; Smith and Wan 2019; Fernandez et al. 2020). Oak root litter utilized in this study was lower quality or had a higher C:N ratio (~41) compared to both maple root (~37) and fungal litter (~8). Although not measured in this study, oak litter is known to contain increased concentrations of tannins (Talbot et al. 2008; Sun et al. 2018) which can complex with organic N compounds and slow decay (Hättenschwiler and Vitousek 2000; Adamczyk et al. 2019). It is possible that tannin-rich oak litter formed recalcitrant complexes with organic N from decaying roots and fungi, slowing decomposition. Greater tannin contents in oak relative to maple litter may have contributed to decay differences in oak and maple plots. Furthermore, these complexes may play an important role in soil C stabilization (Adamczyk et al. 2019).

The labile nature of fungal hyphae (6 X more N per unit C) when compared with the root litter may explain the differential effects of living roots on root and fungal decay in this study. We detected a suppressive effect of maple roots on fungal mass loss after a year and though not statistically significant, the effect of oak roots showed a similar trend. The fungal hyphae used in this study contained significant concentrations of the chemically complex pigment, melanin (Fernandez and Koide 2014). It is possible that at this stage in decay, the fraction of fungal mass remaining was resistant to decay and there was a shift in the decomposer community to less efficient decomposers (Fernandez and Kennedy 2018). A previous study conducted at this site found that in plots dominated by maple trees but containing EcM-associated understory vegetation, the proportion of EcM fungi colonizing decaying *M. bicolor* fungal hyphae increased through time (between 14 and 92 days; Beidler et al. 2020). Dominant EcM fungal species at the site include *Cortinarius caperatus*, *Hygrophorus sordidus* and *Russula ochroleuca* (Beidler et al. 2020). Genes coding for oxidative enzymes have been detected in the genera *Cortinarius*, *Hygrophorus*, and *Russula* (Bödeker et al. 2009); moreover *Cortinarius* spp. have been associated with the degradation of complex organic matter in later stages of decay (Bödeker et al. 2014). To account for decomposer community shifts associated with substrate quality, we suggest that future studies testing 'Gadgil effects' on fungal decay include increased sampling

frequency to capture both shorter- and longer-term time intervals (Maillard et al. 2021).

Limitations and conclusions

We found that root production and mycorrhizal colonization were responsive to belowground litter decay and related to rhizosphere enzyme activity. However, we only measured the foraging behavior of two tree species. To develop a generalizable understanding of belowground nutrient acquisition strategies, more studies are needed to test for trait coordination between roots and root-associated microbes for a greater diversity of plant species (Chen et al. 2018; Sun et al. 2021). We found that both oak and maple tree roots can have a suppressive effect on decay and that the magnitude of this effect was greater for low-quality root litter decaying in N limited mineral soils. A caveat of our design is that we pruned roots to ensure that they were actively growing and placed them in root-free, sieved soils which may have influenced both root responses to and effects on litter decay. Root pruning may result in compensatory growth responses including increases in specific root length or root branching intensity (Feng et al. 2021), as well as enhanced physiological activity of remaining roots (Vysotskaya et al. 2004). Our overall percent root recovery to pruning was similar to previous studies that have utilized root ingrowth bags (~70%; Eissenstat et al. 2015; Liu et al. 2015); however, it is unclear how fine root responses to pruning (a proxy for root herbivory) might influence the activity of mycorrhizal fungi or competitive dynamics between roots and soil microorganisms. This question is ripe for exploration in future studies.

In addition, our experimental design did not allow us to separate the effects of roots vs fungal hyphae on belowground litter decay. More field studies are needed to disentangle root vs fungal contributions to the suppression of decay and may be accomplished using different sized mesh bags to exclude either roots or fungal hyphae (see Lin et al. 2019); a technique that can be especially powerful when paired with microbial community and enzyme analysis (see Maillard et al. 2021). Despite these limitations, our findings demonstrate the potential for the Gadgil effect to operate on root and fungal inputs in temperate forests and to contribute to the persistence of root and fungal derived C in forest soils. Given the known sensitivity of root-microbe interactions to global change drivers (Terrer et al. 2016) and their potential role in forest adaptation to changing environments (Jo et al. 2019), we need to refine our understanding of what controls the direction and extent of root effects on ecosystem processes.

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Author contribution statement Both KVB and RPP conceived of and designed the study. KVB and YEO collected the data. SGP helped with root trait measurements and data interpretation. KVB. wrote the paper with input from all authors.

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Data availability Data and code available on request from the authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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