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Research

Decelerated carbon cycling by ectomycorrhizal fungi is controlled by substrate quality and community composition

Christopher W. Fernandez[1], Craig R. See[2] and Peter G. Kennedy[1,2]

[1]Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108, USA; [2]Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, MN 55108, USA

Author for correspondence:
Christopher W. Fernandez
Tel: +1 507 389 5069
Email: cwfernan@umn.edu

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Summary

- Interactions between symbiotic ectomycorrhizal (EM) and free-living saprotrophs can result in significant deceleration of leaf litter decomposition. While this phenomenon is widely cited, its generality remains unclear, as both the direction and magnitude of EM fungal effects on leaf litter decomposition have been shown to vary among studies.
- Here we explicitly examine how contrasting leaf litter types and EM fungal communities may lead to differential effects on carbon (C) and nitrogen (N) cycling. Specifically, we measured the response of soil nutrient cycling, litter decay rates, litter chemistry and fungal community structure to the reduction of EM fungi (via trenching) with a reciprocal litter transplant experiment in adjacent *Pinus*- or *Quercus*-dominated sites.
- We found clear evidence of EM fungal suppression of C and N cycling in the *Pinus*-dominated site, but no suppression in the *Quercus*-dominated site. Additionally, in the *Pinus*-dominated site, only the *Pinus* litter decay rates were decelerated by EM fungi and were associated with decoupling of litter C and N cycling.
- Our results support the hypothesis that EM fungi can decelerate C cycling via N competition, but strongly suggest that the 'Gadgil effect' is dependent on both substrate quality and EM fungal community composition. We argue that understanding tree host traits as well as EM fungal functional diversity is critical to a more mechanistic understanding of how EM fungi mediate forest soil biogeochemical cycling.

Introduction

Soil organic matter (SOM) decomposition is a critical nexus in the global cycling of carbon (C) and nitrogen (N), and a flux with cascading effects on a range of important ecosystem services, including nutrient availability and soil C stabilisation (Schlesinger and Bernhardt, 2013). In forests, soil fungi are the primary agents of decomposition through the production of extracellular enzymes that break down SOM to acquire growth-limiting resources (Baldrian, 2017). The two dominant fungal guilds involved in forest soil SOM decomposition are free-living saprotrophic fungi and symbiotic ectomycorrhizal (EM) fungi (Lindahl & Tunlid, 2015). These fungi potentially compete with each other as well as other soil biota for resources found in SOM. Unlike saprotrophs, however, EM fungi are not limited by the C in SOM, as it is provided by their tree hosts in the form of simple sugars (Smith & Read, 2008), potentially leading to superior N use efficiency (Smith & Wan, 2019). This is thought to allow EM fungi to allocate resources towards exploiting soil nutrient patches, particularly N, which can be scarce in temperate and boreal forest soils (Kaye & Hart, 1997). The resultant 'N mining' (Kuyper, 2017) by EM fungi would increase the C : N ratio of SOM substrates, limiting saprotrophic growth as those decomposers become increasingly N limited. This scenario creates a positive feedback loop, ultimately resulting in the accumulation of C stored in soil (Gadgil & Gadgil, 1971). This phenomenon, referred to as the 'Gadgil effect', has received renewed interest due to the potential of soil C storage to counteract increases in atmospheric CO_2 concentrations (Orwin *et al.*, 2011; Averill & Hawkes, 2016).

Despite widespread reference to this phenomenon in the literature, knowledge about the generality of the 'Gadgil effect' remains limited (Fernandez & Kennedy, 2016). In particular, field-based experiments implementing EM fungal reduction treatments (e.g. soil trenching and tree girdling) in different forest systems have generated inconsistent results, calling into question the ubiquity of this phenomenon. One of the most important yet poorly understood biotic factors that may modulate the direction and magnitude of the 'Gadgil effect' is soil fungal community composition. With methodological advances in characterising fungal communities (Nilsson *et al.*, 2018), examining the effects of specific EM fungal and soil saprotrophic taxa may provide greater insight into how fungal–fungal interactions mediate rates of SOM decomposition. For example, there is growing evidence that members of the EM fungal genus *Cortinarius*, which possess a range of class-II peroxidase genes,

can significantly alter soil C stocks (Bödeker *et al.*, 2014; Kyaschenko *et al.*, 2017; Sterkenburg *et al.*, 2018). Additionally, many EM fungal species are strongly host specific, and different tree species in close proximity often have dramatically different associated EM fungal communities (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008; Walker *et al.*, 2014). Because of the functional diversity among EM fungi, particularly in their ability to explore and breakdown SOM (Kohler *et al.*, 2015; Pellitier & Zak, 2018), variation in the composition of these communities may have strongly contrasting effects on forest C and N cycling (Zak *et al.*, 2019). Similarly, taxonomic and functional variation among saprotrophic fungi may also influence the direction and magnitude of the 'Gadgil effect' (Van der Wal *et al.*, 2013).

Along with differences in fungal community composition, variation in substrate chemistry is another key biotic variable that may influence how EM fungi affect SOM decomposition rates. Litter chemistry varies considerably across tree species (Berg, 2000; Hobbie, 2008; Phillips *et al.*, 2013), and both local and global analyses indicate that multiple chemical components are closely linked to decomposition rate, particularly N and lignin (Melillo *et al.*, 1982; Hobbie, 2005; Cornwell *et al.*, 2008). As competition for N is the most commonly cited mechanism by which EM fungi may suppress saprotrophic decomposition (see Fernandez & Kennedy, 2016 for a discussion of alternative mechanisms), SOM with low N content may be particularly susceptible to this effect. Consistent with this prediction, EM fungal suppression of litter decomposition has been most pronounced in systems dominated by conifer trees (Fernandez & Kennedy, 2016), which typically have lower litter N content than angiosperm trees (Hobbie, 2005). In addition, there is growing evidence of 'home field advantage' (HFA) effects (Gholz *et al.*, 2000), where litter decomposition is enhanced when litter and canopy composition are matched (Austin *et al.*, 2014; Midgely *et al.*, 2015). The mechanism(s) for HFA effects likely involve more than just optimisation of microbial communities for specific substrates, as factors such as litter nutrient content alone do not capture their full magnitude (Vivanco & Austin, 2008). In fact, competitive interactions among different microbial guilds have been suggested to be important mediators of HFA effects (Van Der Wal *et al.*, 2013). Although there has been some effort to disentangle the effects of litter type and fungal interactions in tropical forests (McGuire *et al.*, 2010), no studies to date have varied litter type and exclusion of EM fungi in the higher latitude forests where a 'Gadgil effect' has been most consistently observed.

The objective of this study was to understand how contrasting host tree and associated EM fungal communities may lead to differential effects on leaf litter decomposition and soil N cycling. Based on widespread presumption in the 'Gadgil effect' literature (e.g. Orwin *et al.*, 2011; Averill *et al.*, 2014), we hypothesised that EM fungi would be correlated with reduced soil N availability and the suppression of litter decay rates would be associated with reduced litter N content in plots where EM fungi were abundant. Based on hypotheses proposed in Fernandez & Kennedy (2016) and more recent modelling work by Smith & Wan (2019), we further hypothesised that given relatively low litter quality of *Pinus* litter (high lignin : N), that a 'Gadgil effect' would be more pronounced in this litter type than in *Quercus* litter. Finally, we speculated that inhibition of litter decomposition would occur primarily in association with EM fungal taxa known to produce enzymes associated with SOM decomposition. To test these hypotheses, we conducted two litter bag decomposition experiments employing a soil trenching treatment, which disrupts host C flow into the plots and reduces EM fungal in-growth, in adjacent *Pinus* and *Quercus*-dominated forest sites in Minnesota, USA. We began by assessing the effectiveness of the trenching treatment of reducing soil EM fungal abundance and EM root in-growth at the sites. We then followed up by examining the influence of EM fungi and EM roots on the availability of soil N and P. In the first litter bag experiment, we sought to establish the presence and consistency of the 'Gadgil effect' in our study system. In the following experiment, we used a reciprocal transplant design where both *Pinus* and *Quercus* litter were independently incubated in the same EM trenching treatments in *Pinus* and *Quercus*-dominated forest sites to assess if potential interactions between fungal community structure and litter type may ultimately govern decomposition dynamics. The composition of the entire fungal community in the soils and colonising the incubated litters in untrenched (control) and trenched plots was assessed using high-throughput sequencing (HTS). Changes in litter mass, C, lignin, and N content of the incubated litter were assessed to determine the effects that EM fungi had on the incubated litter chemistry.

Materials and Methods

Experimental design

Both experiments were conducted at the Cedar Creek Ecosystem Science Reserve in east-central Minnesota, USA. We located plots in two sites based on tree host composition, one being dominated by Northern pin oak (*Quercus ellipsoidalis*) (45.42142N, 093.19509W) (hereafter referred to as the oak site) and the other being dominated by Eastern white pine (*Pinus strobus*) (45.42577N, 093.20852W) (hereafter referred to as the pine site) (Supporting Information Table S1). These sites are located *c.* 1 km apart and have the same underlying sandy poorly developed Udipsamment soils, which have comparable soil pH and inorganic nitrogen concentrations within each soil layer (Table S2). On 8 June 2015, six randomly located blocks were established in each site, each containing a untrenched and a trenched EM fungal reduction treatment. The blocks were located at least 8 m apart to avoid spatial autocorrelation in fungal community composition (Lilleskov *et al.*, 2004; Bahram *et al.*, 2012). Trenching was done using a spade and cutting to a depth of 30 cm, which severed root and EM fungal in-growth into the plots. Given the newly generated inputs of labile carbon substrates (i.e. dead roots and fungal mycelium), the trenched plots were allowed to equilibrate after the initial disturbance for 5 wk before commencing with the first experiment. To reduce root and EM fungal growth into the trenched plots, we carefully re-ran the spade through the trench slits on a bi-weekly basis during the growing season (July–

November 2015, April–November 2016, April–July 2017). We measured the effect of trenching on mineral soil moisture by taking soil cores (the same used later in molecular analyses) from each plot and determining the gravimetric water content on 5 g subsamples. We also monitored the effect of the trenching treatment on O-layer moisture content at five time points in the first growing season (July–November 2015) by collecting O-layer material and determining gravimetric moisture content from adjacent untrenched and trenched plots established for this purpose.

Litter decomposition

Because forest soils at Cedar Creek are generally composed of unfragmented leaf litter directly above the soil A-layer, we used leaf litter as our substrate source, matching the original 'Gadgil effect' experiment (Gadgil & Gadgil, 1971) and the majority of studies that followed (Gadgil & Gadgil, 1975; Berg & Lindberg, 1980; Staaf, 1988; Zhu & Ehrenfeld, 1996; Koide & Wu, 2003; Mayor & Henkel, 2006; McGuire *et al.*, 2010; Brzostek *et al.*, 2015; Sterkenberg *et al.*, 2018). Recently senesced *Pinus strobus* and *Quercus ellipsoidalis* leaf litter (hereafter referred to as pine and oak litter, respectively) were collected from each forest type in October 2014. Both litter types were brought back to the laboratory and dried at 50°C for 48 h. After drying, the litters were carefully sorted to remove other organic matter (e.g. twigs) and then stored at room temperature in paper bags before litter bag construction. Here, *c.* 2 g of oven-dried pine or oak litter was weighed and placed into litter bags constructed of polyurethane 2 mm mesh (Industrial Netting, Minneapolis, MN, USA; Product #XN3234) *c.* 12 × 12 cm in dimension and heat sealed closed. For the first experiment, litter bags matching thee canopy composition of the forest (i.e. pine litter in the pine site and oak litter in the oak site) were incubated at the soil–litter layer interface in each plot, with the incubation starting on 15 July 2015. For the second experiment, both pine and oak litter (in separate litter bags) were incubated in the same plots, with the incubation commencing on 15 July 2016. In each experiment, the litter bags were incubated in each plot for 2, 4 or 12 months. Upon harvesting, the litter bags were placed in sterile plastic bags and transported back to the laboratory where the litter was re-dried at 50°C until the mass was stable. The remaining mass of the decomposed litter was determined using plastic weighing trays sterilised with 70% ethanol. The dried litter was then stored in labelled sterile plastic bags at −20°C ahead of elemental and molecular analyses.

Litter chemistry

Lignin, cellulose, and hemicellulose concentrations were measured for initial and 12-month incubated litter using an ANKOM Fiber Analyzer (Ankom Technology, Macedon, NY, USA; Hobbie, 2008). The C and N content for samples incubated for 12 months was assessed via dry combustion (Costech ECS 4010 Elemental Analyzer, Valencia, CA, USA) at the University of Minnesota. Elemental contents of the mass remaining were then calculated by multiplying mass by concentration.

Fine root in-growth

To determine the effectiveness of the trenching treatment in terms of reduction of fine root in-growth, we placed in-growth cores (5 × 15 cm) containing sieved soil just inside and outside the edge of the trenched plots in each block and incubated them for 30 d in July 2016. The in-growth cores were brought back to the laboratory and the soil was sieved with a 2 mm sieve to collect total root biomass. Root biomass was then rinsed in water and fine roots were identified as herbaceous or woody. Only fine woody roots, which dominated the root pool, were dried, weighed and included in the analysis.

Soil nutrient availability

To assess soil inorganic nutrient availability in the untrenched and trenched plots, we incubated three pairs of plant root simulator (PRS) probes (Western Ag Innovations; Saskatoon, SK, Canada) in each plot from 2 June to 6 July 2017, which corresponds to a period of high plant productivity at Cedar Creek. The cation and anion PRS probes were oriented vertically in the top 10 cm of the soil and across the soil plots. After the incubation the PRS probes in each plot were pooled and sent to Western Ag Innovations for processing.

Fungal community identification

Genomic DNA was extracted from all litter samples using MoBio PowerSoil kits (MoBio, Carlsbad, CA, USA). Before extraction, a *c.* 100 mg subsample was homogenised via bead beating in 2 ml tubes containing three 1 mm zirconia/silica beads (BioSpec Products, Bartlesville, OK, USA). In addition, genomic DNA from soil cores collected from untrenched and trenched plots 12 months after establishment were also extracted using the same extraction method. To characterise fungal community composition, rDNA of the ITS1 region was PCR amplified using a barcoded fungal-specific ITS1F-ITS2 primer set, following the reagent and cycling conditions detailed in Smith & Peay (2014). While this primer set has been critiqued for not amplifying members of the saprotrophic fungal genus *Mycena* (Tedersoo & Lindahl, 2016), we found many sequences that could be successfully matched to this genus, so do not think this primer choice resulted in significant methodological bias. A 25 fungal species mock community detailed in Nguyen *et al.* (2015) was also included. Amplified products were cleaned and normalised individually using Charm 'Just-a-Plate' kits (Charm, San Diego, CA, USA). The samples from each experiment were pooled into individual libraries and sequenced at the University of Minnesota Genomics Center using 250-bp paired-end V2 MiSeq Illumina chemistry (Illumina, San Diego, CA, USA).

Fungal sequences were processed using the AMPTK pipeline v.1.1 (Palmer *et al.*, 2018). Briefly, the forward and reverse sequences in each sample were demultiplexed, the primers

removed, and then denoised using the UNOISE3 algorithm (Edgar, 2016). The resulting 'inferred sequences' (a.k.a. exact sequence variants) were clustered into operational taxonomic units (OTUs) at 97% similarity using VSEARCH (Rognes *et al.*, 2016). Taxonomy was assigned using a 'last common ancestor' approach of global USEARCH, UTAX and SINTAX alignments against the UNITE v7.2.2 database (Kõljalg *et al.*, 2013). To remove possible sequences caused by index bleed, a 0.5% filtering was applied to each sample. In addition, for each OTU, any sequence reads present in the PCR negative controls were subtracted from read abundances present in the litter samples (Nguyen *et al.*, 2015). Finally, the mock community was also used to determine the level at which unexpected sequence reads were encountered. From this, it was determined that a four sequence read cutoff should be used to remove spurious OTUs, resulting in all cells with values less than four sequence reads being zeroed.

Following Sterkenberg *et al.* (2015), we assigned all OTUs belonging to the Eurotiales, Hypocreales, Morteriellales, Mucorales, Saccharomycetales, Tremellales and Sporidiales as 'Moulds & Yeasts' to better reflect the r-selected life history strategies of these groups and distinguish them from soil and litter associated saprotrophic fungi. The remaining OTUs were assigned to saprotrophic, ectomycorrhizal, other symbiotrophic (e.g. arbuscular and ericoid mycorrhizal fungi), and pathotrophic guild modes using FUNGUILD (Nguyen *et al.*, 2016). When possible, the top 50 most abundant unassigned OTUs (due to missing genus taxonomy) were assigned manually to either EM or saprotrophic guilds using criteria detailed in Fernandez *et al.* (2017). A list of guild assignments is provided in Table S3. From the 4294 076 sequence reads passing the quality filtering steps, 2423 625 sequence reads (mean = 55% of reads/sample) could be assigned to functional guilds. Because of variation in sequence read depth per sample, the final dataset was normalised to proportional abundances or Hellinger transformed (sqrt relative abundance) before analysis. Raw sequence read files are available in NCBI SRA accession: soil (PRJNA560603), Experiment 1 litter (PRJNA560605), Experiment 2 litter (PRJNA560606).

Statistical analyses

The effect of site, trenching and potential interactions on root in-growth rates, soil moisture, soil fungal guild abundance, soil nutrient availability, and incubated litter chemistry (12 months) were assessed using linear mixed models with block nested in site as a random factor. For Experiment 1, the effect of trenching and incubation time on mass remaining was tested with linear mixed models for each litter type with block nested in site as a random effect. For Experiment 2, the effects of site, trenching, and incubation time on litter mass remaining were tested using linear mixed models for each of litter type with block nested within site as a random effect. All mixed models were run using the 'lme' function in NLME package in R. Soil nutrient availability data and all litter mass remaining data was $\log_e$ transformed before analysis to satisfy the linearity assumptions. To visualise HTS fungal community data we used 'amp_ordinate' functions in the AMPVIS2 package in R to construct nonmetric multidimensional scaling plots. The effects of trenching and incubation time on fungal community composition were assessed using factorial permutational analyses of variance (PERMANOVA). Before each PERMANOVA, data were Hellinger-transformed and pair-wise distances were calculated based on Bray–Curtis dissimilarity. To further assess whether significant results detected by the PERMANOVA analyses were due to shifts in composition or heterogeneity, betadisper tests were run on significant predictor variables. Finally, analyses of covariance (ANCOVAs) were used to detect the effect of the trenching treatment on the C and lignin content per unit N for each of the litter types after 12 months of incubation.

Results

Soil

Soil fungal community composition differed significantly among the two sites (Fig. 1a, PERMANOVA; Site: $F_{1,42} = 12.43$; $P < 0.001$), which was not caused by differences in community heterogeneity (betadisper: Site: $P = 0.670$). EM fungi dominated the soil fungal communities at both sites (Fig. 2). The most abundant EM fungal genera in the pine site were *Tomentella*, *Russula* and *Inocybe* (Fig. 1b), whereas *Russula*, *Amanita*, *Scleroderma*, *Tomentella*, *Cortinarius* and *Cenococcum* were the dominant EM fungal genera in the oak site (Fig. 1c). The non-mycorrhizal (i.e. saprotrophic, moulds and yeasts, pathotrophic) fungal genera dominating the pine site soil community included *Lepiota*, *Chalara*, *Trechispora* and *Mortierella,* while *Mortierella, Cladophialophora,* and *Vararia* dominated the oak site soil communities (Fig. 1c). Trenching had a marginal effect on fungal community composition (PERMANOVA; Trenching: $F_{1,42} = 1.37$, $P = 0.108$), but significantly reduced the relative abundance of EM fungi in soils in both sites (Trenching: $F_{1,30} = 11.37$, $P = 0.002$; Fig. 2). By contrast, trenching increased the relative abundance of moulds & yeasts in both sites (Trenching: $F_{1,30} = 18.70$, $P < 0.001$), and saprotrophic fungi in the pine site but not the oak site (Site × Trenching: $F_{1,30} = 6.05$, $P = 0.02$; Fig. 2). While the trenching treatment did not completely reduce the relative abundance of EM fungi, this was expected due to the persistence of relic DNA in soils (Carini *et al.*, 2017).

Root in-growth rates were approximately twice as fast in the oak site compared to the pine site, based on measurements in the untrenched plots (Fig. S1). Trenching significantly reduced root in-growth frequency and decreased mean in-growth rate in both sites (Trenching: $F_{1,10} = 8.67$; $P = 0.014$; Fig. S1). There were no significant differences between the O-layer (Site: $F_{1,9} = 0.04$; $P = 0.840$) and mineral soil moisture (Site: $F_{1,9} = 1.73$, $P = 0.240$) between the two sites. Trenching increased mineral soil moisture from 3.6% to 5.9% water on average (Trenching: $F_{1,10} = 22.17$; $P = 0.001$). Although trenching significantly reduced root in-growth rates and mineral soil moisture at both sites, there was no effect of this treatment on O-layer moisture content in either site during the growing season (Trenching:

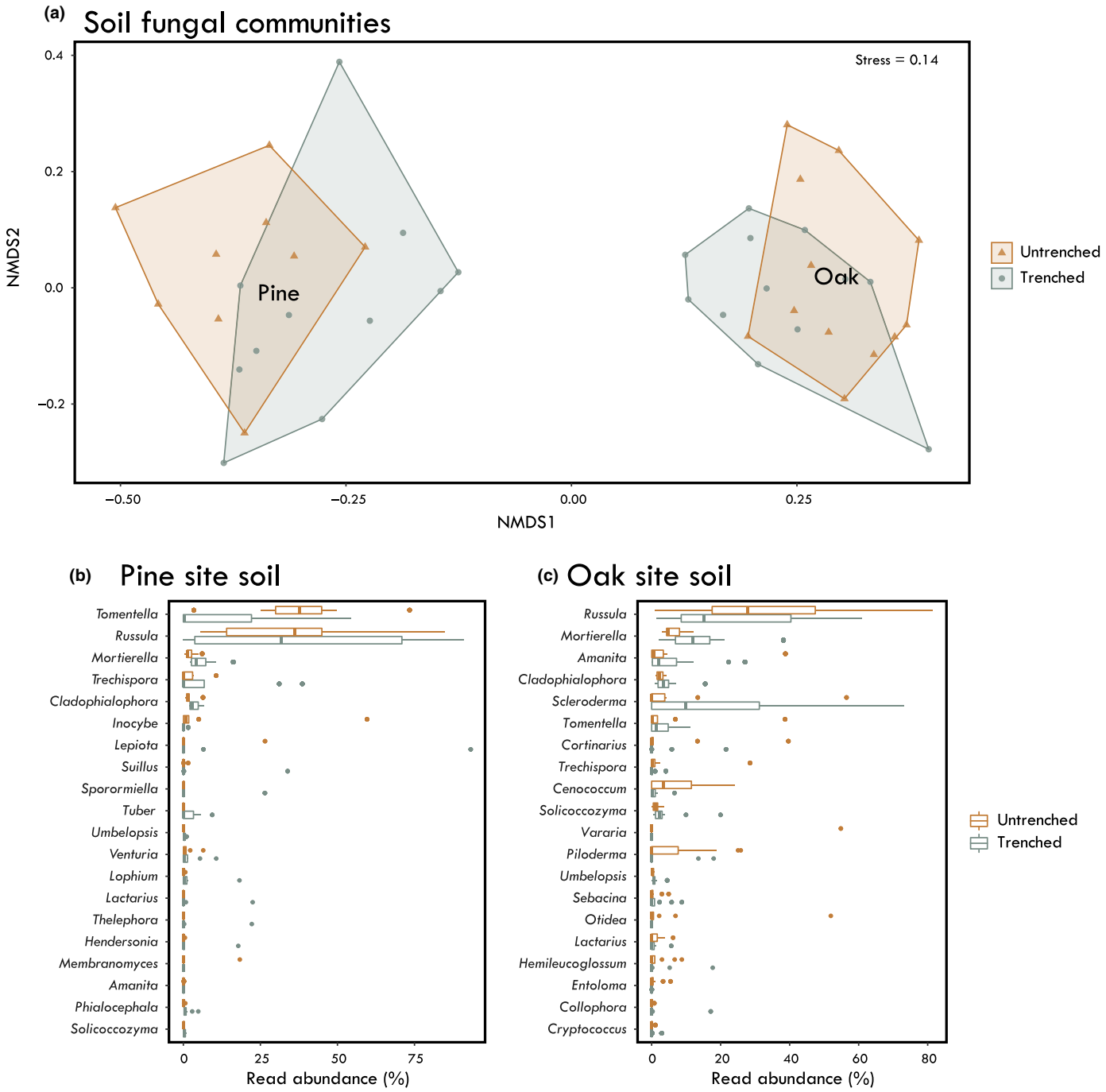

Fig. 1 Soil fungal communities at the oak and pine sites and their response to trenching. Bray–Curtis Dissimilarity based nonmetric multidimensional scaling (NMDS) plots of soil fungal communities present in the oak and pine sites. Each site label represents the site centroid (a). Samples (circles) and frames are coloured by untrenched (orange) and trenched (grey) treatments to help visualise compositional differences. Boxplots of relative read abundance of the top 20 most abundant fungal genera present in the in untrenched (orange) and trenched (grey) treatments in the pine site soils (b) and the oak site soils (c). Frames represent the range of each treatment and site in ordination space ($n = 44$).

$F_{1,10} = 0.04$, $P = 0.837$). EM root in-growth rate was positively correlated with EM fungal relative abundance in both sites (Fig. S2). Conversely, EM root in-growth rate was negatively correlated with saprotrophic fungal relative abundance in the pine site but no relationship was detected in the oak site (Fig. S2). With respect to inorganic N availability, the trenching significantly increased soil N availability in the pine site but had no effect in the oak site (Site × Trenching: $F_{1,10} = 6.48$; $P = 0.029$; Fig. 3). Conversely, phosphorous availability was significantly higher in the untrenched plots in both sites (Trenching: $F_{1,10} = 6.75$; $P = 0.026$; Fig. 3).

EM root in-growth rates were marginally negatively correlated with soil N availability in the pine site ($P = 0.10$; $R^2 = 0.26$), but no significant relationship was detected in the oak site (Fig. 4a).

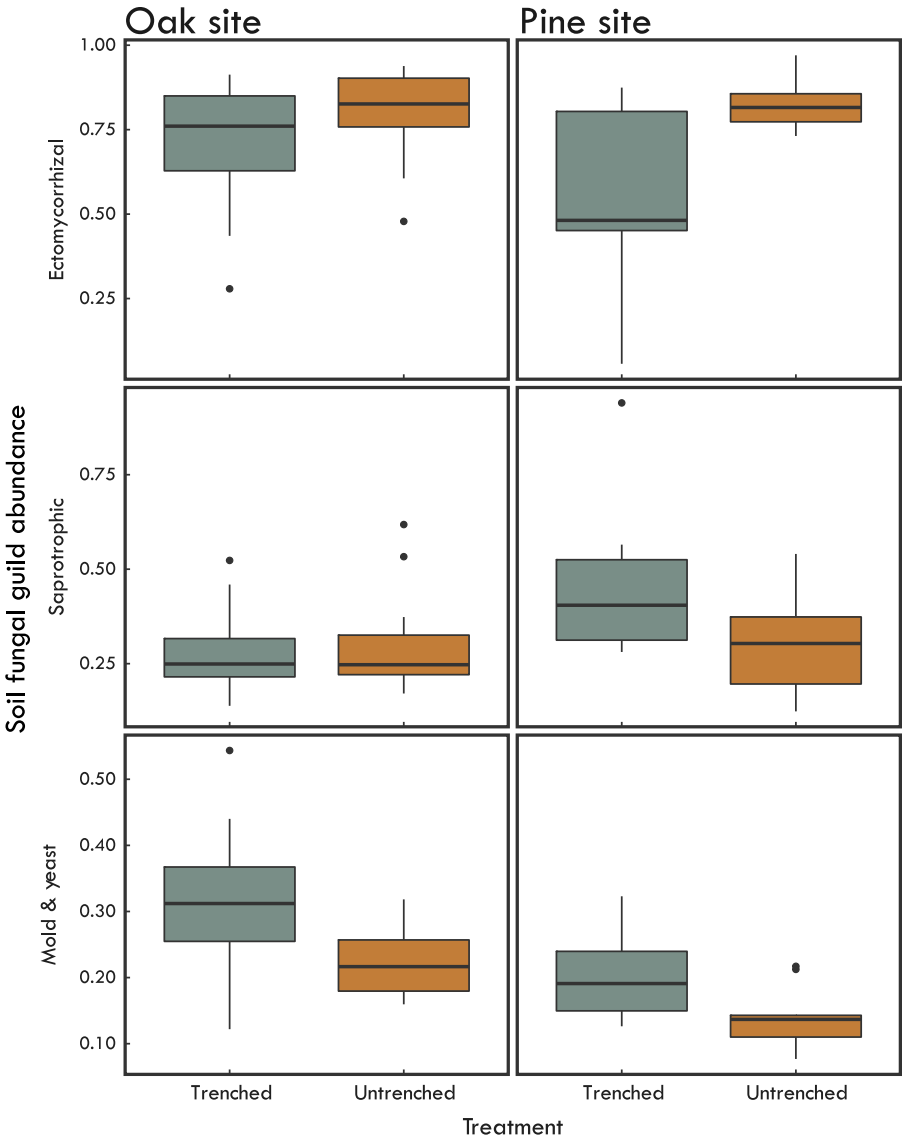

Fig. 2 Boxplots of ectomycorrhizal, saprotrophic, and mould & yeast abundance (based on Hellinger-transformed sequence read counts) in the untrenched (orange) and trenched (grey) treatments after 12 months oak and pine site soils. Boxes correspond to median (line in box), 25 percentile (lower hinge) and 75 percentile (upper hinge) ranges. Whiskers extend from 25 and 75 percentile limits to the smallest and largest values that do not exceed 1.5 × interquartile range limit in each direction. Data beyond the end of the whiskers are plotted individually as circles ($n = 44$).

Similarly, the ratio of ectomycorrhizal-to-saprotrophic fungal abundance was negatively correlated with soil N availability in the pine site ($P = 0.008$; $R^2 = 0.56$), but again no significant relationship was detected in the oak site (Fig. 4b).

Litter decomposition

Experiment 1 There was no effect of trenching on oak litter mass loss rates (Table 1). In fact, litter mass loss rates in the trenched oak site plots were actually lower at the 2 month time point (Fig. S3), suggesting that, at least initially, roots and/or EM fungi may be involved in accelerating litter decomposition rather than suppressing it in this site. Conversely, the trenching treatment had an increasingly positive effect on decomposition rates of pine litter incubated in the pine site (Table 1; Fig. S3).

Experiment 2 The effect of trenching on the mass loss was different for the two litter types and also depended on the site in which it was incubated (Table 2). Trenching significantly increased pine litter mass loss rates when incubated in the pine site after 12 months (Fig. 5d), but not when pine litter was incubated in the oak site (Fig. 5c). By contrast, oak litter mass loss rates were not significantly affected by trenching in either site (Table 2; Fig. 5a,b). Oak litter, which had lower lignin and higher N concentrations (Table 3), had, on average, higher rates of mass loss compared to pine litter, after 12 months of decomposition. However, litter mass loss rates were, on average, higher in the pine site relative to the oak site.

The N concentration of the incubated litter differed significantly depending on litter type and the site in which it was incubated (Site × Litter: $F_{1,28} = 7.63$, $P = 0.010$). This interaction was driven by oak litter, which had a higher initial N concentration compared to pine litter (Table 2), but had significantly lower postincubation N concentrations than pine litter when decomposed in the pine site regardless of trenching (Table S4; Fig. S4). There were no significant effects of trenching on litter N concentration (Table S4), but the C : N ratios of the incubated litters

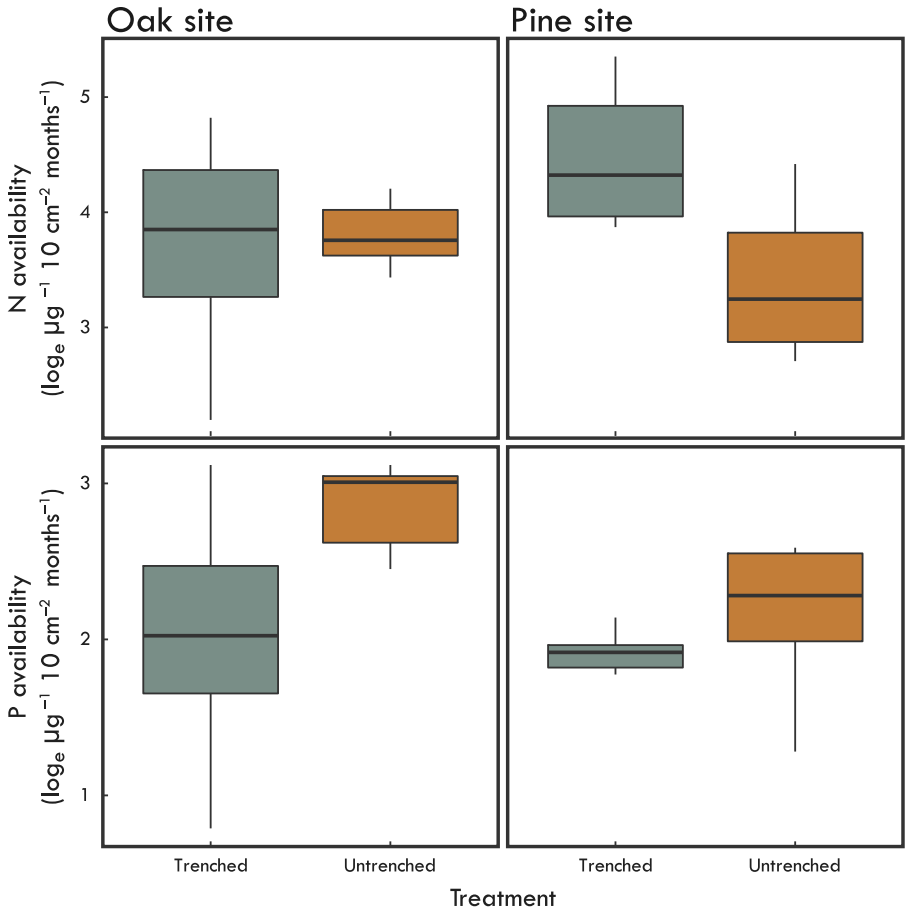

Fig. 3 Soil nitrogen (N) and phosphorus (P) availability in the untrenched (orange) and trenched (grey) treatments after 12 months oak and pine sites soils. Boxes correspond to median (line in box), 25 percentile (lower hinge) and 75 percentile (upper hinge) ranges. Whiskers extend from 25 and 75 percentile limits to the smallest and largest values that do not exceed 1.5 × interquartile range limit in each direction. Data beyond the end of the whiskers are plotted individually as circles ($n = 24$).

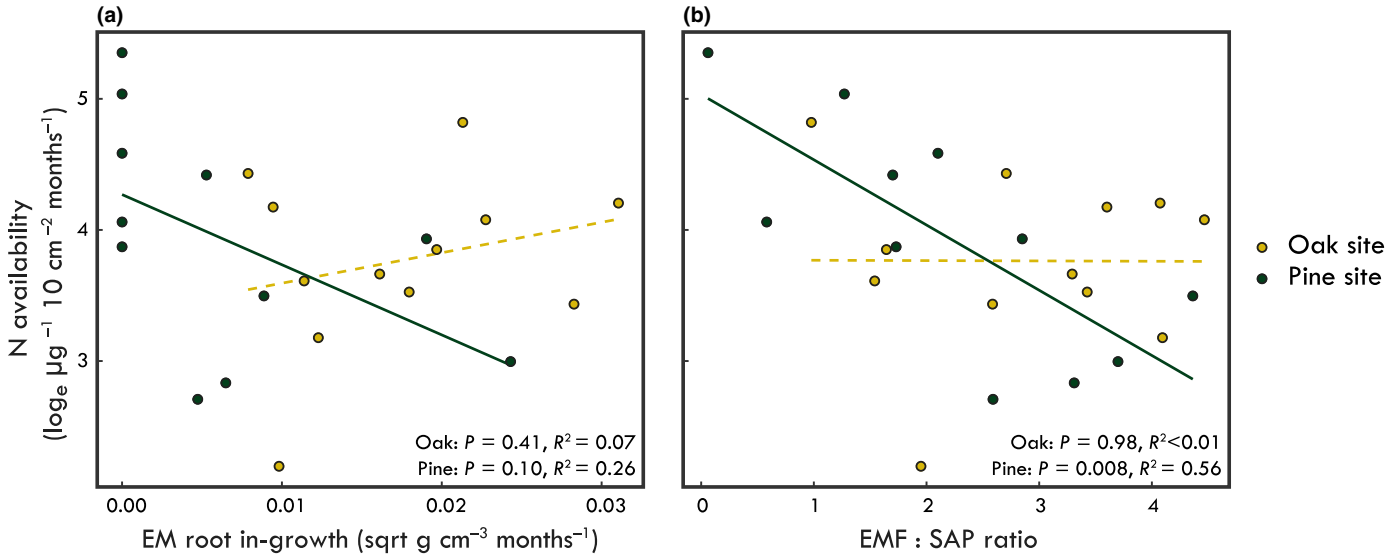

Fig. 4 Relationship between soil nitrogen (N) availability and ectomycorrhizal root in-growth rate (a); and soil ectomycorrhizal-to-saprotrophic abundance ratio (EMF:SAP) (b). Data points and regression lines are coloured by oak (yellow) and pine (green) site soils. Both untrenched and trenched treatments were included in the analyses ($n = 23$).

were higher in the untrenched compared to trenched plots (Trenching: $F_{1,28} = 4.54$; $P = 0.042$; Fig. S5). In addition, there was an interaction between litter type and site; each of the litters had slightly higher C : N ratios when incubated in the site with nonmatching canopy composition (Site × Litter: $F_{1,28} = 9.31$; $P = 0.005$).

Table 1 Experiment 1 effects tests from mixed models explaining litter mass remaining.

Litter type and site	Fixed effects	df num	df den	*F*	*P*
Oak	(Intercept)	1	27	28039.54	<.0001***
	Incubation	1	27	44.381	<.0001***
	Treatment	1	27	0.515	
	Incubation × Treatment	1	27	0.196	0.6613
Pine	(Intercept)	1	27	20198.63	<.0001***
	Incubation	1	27	12.075	0.0017**
	Treatment	1	27	5.589	0.0255*
	Incubation × Treatment	1	27	1.303	0.2636

Asterisks indicate statistical significance at the level of: ***, $P<0.0001$; **, $P<0.01$; *, $P<0.05$.

While the C concentration of the incubated litters largely had a consistent response to trenching within sites, more detailed analysis of the C remaining after incubation revealed important differences in the recalcitrant lignin fraction in response to trenching among the litter types and sites (Site × Litter × Trenching: $F_{1,28}=5.14$; $P=0.031$). Pine litter always had higher lignin concentrations and lignin : N ratios in untrenched plots compared to trenched plots no matter which of the sites in which it was incubated (Figs S4, S5). Conversely, oak litter incubated in the oak site had significantly lower lignin concentration in the untrenched plots compared to trenched plots, but when incubated in the pine site the opposite was observed (Fig. S4). Lignin loss during the incubation was also affected by a litter by treatment interaction ($P<0.001$), with pine litter losing 14% and 15% *more* lignin in the trenched than untrenched plots in the oak and pine sites, respectively. Conversely, oak litter lost 15 and 4% *less* lignin in the trenched than untrenched plots in the oak and pine sites, respectively. Litter types also had notably different responses to trenching in terms of remaining C content per unit N (Table S5; ANCOVA: N Content × Litter × Trenching: $F_{1,20}=5.62$; $P=0.003$) as well as remaining lignin content per unit N (Table S5; ANCOVA: N Content × Litter × Trenching: $F_{1,20}=12.20$; $P=0.002$). Pine litter had higher C and lignin content per unit N in the untrenched than trenched plots (Fig. 6b,d), whereas for oak litter there was no difference in terms of C content per unit N by treatment and lower lignin content per unit N in the untrenched than trenched plots (Fig. 6a,c).

Table 2 Experiment 2 effects tests from mixed models explaining oak (a) and pine (b) litter mass remaining.

Fixed effects	df num	df den	*F*	*P*
(a) Oak litter				
(Intercept)	1	53	22810.86	<.0001***
Site	1	5	2.295	0.1902
Incubation	1	53	67.134	<.0001***
Trenching	1	53	2.508	0.1192
Site × Incubation	1	53	5.199	0.0266*
Site × Trenching	1	53	0.85	0.3607
Incubation × Trenching	1	53	1.065	0.3068
Site × Incubation × Trenching	1	53	2.745	0.1035
(b) Pine litter				
(Intercept)	1	54	136236.4	<.0001***
Site	1	5	8.5	0.0332
Incubation	1	54	240.49	<.0001***
Trenching	1	54	1.86	0.1783
Site × Incubation	1	54	10.26	0.0023**
Site × Trenching	1	54	7.28	0.0093
Incubation × Trenching	1	54	8.47	0.0052**
Site × Incubation × Trenching	1	54	0.86	0.3576

Asterisks indicate statistical significance at the level of: ***, $P<0.0001$; **, $P<0.01$; *, $P<0.05$.

Litter and forest types were also important determinants of fungal guild relative abundances over the course of the incubation (Table S6; Fig. S6). Oak litter incubated in both forests was dominated by saprotrophic fungi, although the change in their relative abundance over the incubation varied somewhat between forest types. The relative abundances of all other fungal guilds associated with the oak litter, including EM fungi, were very low compared to saprotrophic fungi (Fig. S6). Conversely, relative abundances of various fungal guilds colonising the pine litter were far more even in both sites and pine litter had notably higher EM fungal colonisation in the untrenched treatment of the pine site (Fig. S6).

The specific taxonomic composition of the fungal communities colonising litter was dependent on site, litter type, and incubation time (PERMANOVA: Site × Litter type × Incubation: $F=1.93$; $P=0.035$), with trenching again having marginal effects (PERMANOVA: Trenching: $F=1.61$; $P=0.070$). Notably, members of the EM genus *Tomentella* were abundant in the pine litter incubated in the untrenched plots in the pine site and were dramatically reduced in the trenched plots (Fig. S7). When the oak litter was incubated in the pine site *Tomentella* was also somewhat abundant after 12 months of incubation, but nowhere near the levels seen in the pine litter (Fig. S7). Conversely, when the pine litter was incubated in the oak site, the EM genus *Amanita* was moderately abundant in the untrenched plots yet practically absent in the trenched plots. Many of the dominant nonmycorrhizal fungi showed litter-specific associations and abundance patterns (e.g. *Talaromyces* and *Pezicula* for oak litter, *Lophium* and *Xenopolyscylalum* for pine litter (Fig. S7), but generally had similar or higher relative abundances in the trenched compared with the untrenched plots.

Discussion

Generality and context dependency of the 'Gadgil effect'

While our combined results support earlier findings that EM fungi can suppress leaf litter decomposition rates, they also question the generality of this phenomenon. In soils at the pine site, we found a clear and consistent negative relationship between EM fungi and N availability. Specifically, when EM fungal ingrowth and abundance were reduced by trenching, there was significantly faster litter decomposition, which is consistent with the 'Gadgil effect'. Conversely, in soils at the oak site, we found

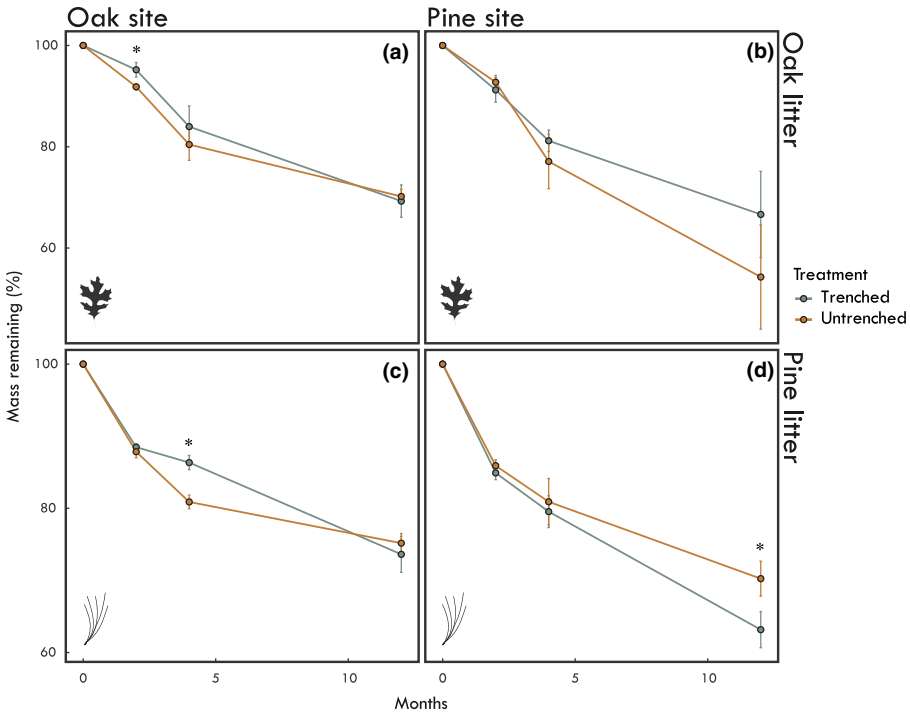

Fig. 5 Per cent mass remaining (mean ± SE) of oak (a, b) and pine (c, d) litter incubated in the untrenched (orange) and trenched (grey) treatments for 2, 4 and 12 months in the oak (a, c) and pine site (b, d) ($n = 196$). Asterisks indicate a significant difference in percent mass remaining between the untrenched and trenched treatments at a given time point as determined by post-hoc *t*-tests ($P < 0.05$).

Table 3 Initial litter chemistry.

	Oak		Pine	
Litter component	%	SE	%	SE
Total carbon	52.4	0.26	53.3	0.31
Total nitrogen	1.20	0.02	0.65	0.01
Hemicellulose	15.6	1.19	14.7	0.53
Cellulose	18.2	1.76	20.3	0.53
Lignin	25.3	0.14	26.7	0.11

neutral relationships between EM fungi and soil N availability, and trenching had no effect on litter decomposition rates. These results, while based at the local scale, parallel inconsistencies observed at larger spatial scales in the literature (Fernandez & Kennedy, 2016). Some experiments have shown strong negative effects of EM fungi on OM decomposition (Gadgil & Gadgil, 1971; Gadgil & Gadgil, 1975; Berg & Lindberg 1980; Fisher & Gosz, 1986; Koide & Wu, 2003; Averill & Hawkes, 2016; Sterkenburg *et al.*, 2018; Maaroufi *et al.*, 2019), while others have reported no effects (Harmer & Alexander, 1985; Staaf, 1988; Mayor & Henkel, 2006; McGuire *et al.*, 2010; Brzostek *et al.*, 2015), or even positive effects (Zhu & Ehrenfeld, 1996). Comparing results across those studies is complicated by the fact that they are conducted in different ecosystems with many covariables (e.g. climate and soil properties), which may interact with the effect of EM fungi on soil biogeochemical cycling. Here, by working in sites in close physical proximity, we held climate and edaphic factors functionally constant, thereby isolating the effects of tree hosts and EM fungal communities on forest C and N cycling. The strong differential responses we observed here among sites differing in both plant and fungal community composition point to the importance of understanding the biotic underpinnings of fungal interguild competition and the 'Gadgil effect'.

Litter-fungal community interactions

Teasing apart the interactions between top-down (litter chemistry) and bottom-up (EM fungi) controls over litter decomposition is necessary for a complete understanding of the context dependency of the 'Gadgil effect'. Fernandez & Kennedy (2016) hypothesised that N availability and narrow C : N ratio of litter inputs may favour higher carbon use efficiency among free-living saprotrophs and reduce the effectiveness of EM fungi in acquiring organic N. This was supported by results from Kyaschenko *et al.* (2017), who found correlative evidence along a fertility gradient that competitive interactions between EM and saprotrophic fungal guilds may be partially driven by N availability. Recently, Smith & Wan (2019) modelled the competitive interactions between EM and saprotrophic fungi and their consequences on soil C and N cycling by applying resource ratio theory (Tillman, 1982) to better understand and predict context dependencies of the 'Gadgil effect'. The model predicted that litter decay rates were decelerated only when the starting substrate was energetically unfavourable to saprotrophic fungi (e.g. wide C : N ratio, high lignin : N ratio). While our results support this predicted top-down control of fungal competitive interactions, they also suggest that EM fungal community composition and inherent functional differences (e.g. enzymatic suites, exploration strategy, vertical niche preference) are probably of equal importance. Specifically, we found that the higher quality oak litter was always dominated by saprotrophs, had low EM fungal colonisation and decay rates that were largely unresponsive to the trenching

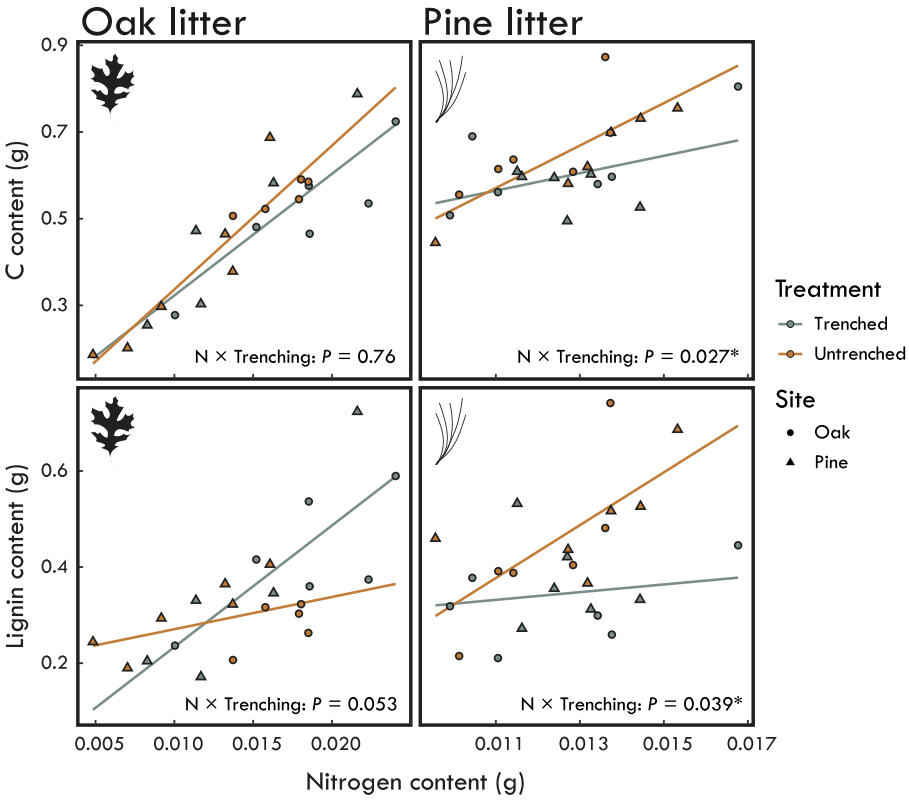

Fig. 6 The relationship between oak and pine litter carbon (C) content and lignin content with nitrogen (N) content by untrenched (orange) and trenched (grey) treatments. Differences in slopes among the treatments were determined with ANCOVA models. (Oak litter $n = 21$; pine litter $n = 23$.)

treatment. Additionally, only the lower quality pine litter (wider initial C : N and lignin : N ratios), when incubated in the pine site was dominated by EM fungi and led to slowed decay rates. Conversely, when pine litter was incubated in the oak site, we observed an opposite trend. Because the two sites differed significantly in fungal community composition, this suggests that there are important functional differences among members of these EM fungal communities. Finally, over the 12 month incubation we observed widening of these ratios in the pine litter in the untrenched plots compared to the trenched plots, suggesting that EM fungi were involved in decoupling of C and N cycling of the litter, which was not observed in the oak litter. Taken together, these findings support a view that EM-mediated deceleration of leaf litter is likely dependent on both substrate chemistry and EM fungal community composition (Fig. 7).

Functional diversity of ectomycorrhizal fungi

Despite the litter/humus layers of soils being frequently thought of as the exclusive domain of saprotrophic fungi (Lindahl *et al.*, 2007), a growing number of studies have demonstrated that EM fungi often colonise these substrates as well (Dickie *et al.*, 2002; Genney *et al.*, 2006; Hobbie *et al.*, 2014; Anderson *et al.*, 2014). Given the functional diversity that exists within and across EM fungal communities (Read & Perez-Moreno, 2003; Finlay, 2008; Koide *et al.*, 2007, 2014), it is not particularly surprising that the litter decomposition patterns we observed imply important functional differences among the EM fungi associated with each forest. Interestingly, however, unlike previous work focusing on the genus *Cortinarius* (Clemmensen *et al.*, 2015; Kyashenko *et al.*, 2017), the suppression of free-living fungi and litter decay rates was consistently associated with increased abundance of EM fungal OTUs in the genus *Tomentella*. Although this genus has not been previously recognised as one with high SOM degradation potential, a number of studies indicate that EM root tips colonised by these fungi are capable of producing a wide range of extracellular enzymes used to break down proteins, polysaccharides, and organic forms of P (Courty *et al.*, 2005; Tedersoo *et al.*, 2012). In addition, Pena *et al.* (2013) demonstrated that *Tomentella badia* colonised ^{15}N-labelled beech leaf litter (*Fagus sylvatica*) and the enrichment of associated EM roots were approximately four times higher than any of the other EM fungi examined. That result indicates that at least some members of this genus are highly capable of mobilising organic N from leaf litter. A second intriguing finding with regard to *Tomentella* litter colonisation was its very limited colonisation of the oak litter incubated in the pine site. Again, we suspect this may in part be due to the oak litter having relatively labile chemistry that favoured free-living fungi (Smith & Wan, 2019). We further hypothesise that the EM fungal community in our oak site may therefore be comprised of EM fungi that favour acquisition of mineral bound N and/or priming of N mineralisation rates via C exudation by roots and EM fungi (Phillips *et al.*, 2012). Finally, it is important to emphasise that while *Tomentella* species appear to be driving a 'Gadgil effect' in this system, other EM fungal taxa may have similar effects and influence on C and N cycling in other systems. We therefore recommend further investigation into EM fungal community composition and linkages with the 'Gadgil effect' in other ecosystems with the hope of identifying common functional traits influencing the phenomenon.

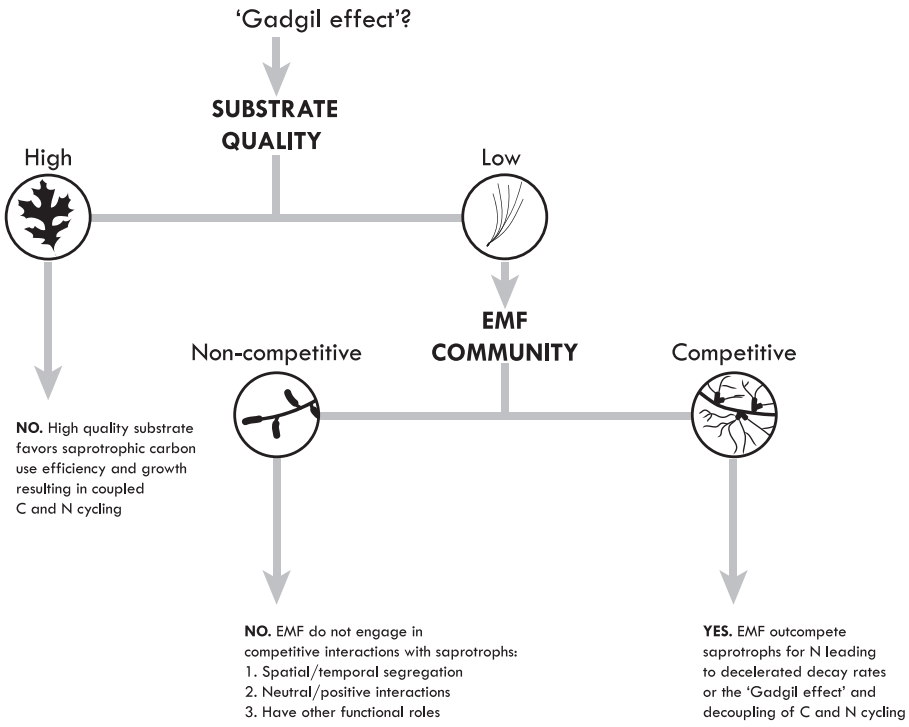

Fig. 7 Conceptual summary of the effects of litter substrate quality and ectomycorrhizal fungal (EMF) community composition on the presence or absence of the 'Gadgil effect'.

While we believe our results provide important insights regarding the role of EM fungi in mediating leaf litter decomposition, a number of caveats should be noted. In our study system (Cedar Creek Ecosystem Science Reserve), the soils lack a well developed organic layer, which may increase the shared realised niche space between EM fungal and saprotrophic guilds and thereby intensifying interactions (Bödeker *et al.*, 2016). As noted above, this possibility was supported by the recent findings of Kyaschenko *et al.* (2017), who showed strong correlative evidence that competition between EM and saprotrophic guilds may be partially driven by the degree of organic layer development. Like other recent studies of the 'Gadgil effect' (Averill & Hawkes, 2016; Sterkenberg *et al.*, 2018), our inferences regarding EM and saprotrophic fungal abundance are based on relative abundances calculated from sequence read counts. While we clearly acknowledge the semiquantitative nature of this metric (Amend *et al.*, 2010), we believe the consistent and sizable declines in EM-to-saprotrophic fungal ratios in the trenched plots and the positive correlation between EM root in-growth indicates the differences in C and N cycling we observed were related to significant changes in EM abundance. Additionally, while the results we observed are consistent with the broader literature noting the preferential presence of a 'Gadgil effect' in conifer forests, further investigation of the phenomenon at larger spatial scales is needed to confirm this pattern. Finally, we recognise that the suppression of litter mass loss we observed does not necessarily translate directly into greater C stocks in soil (Schmidt *et al.*, 2011). For instance, while the suppression of litter and particulate organic matter decay rates by EM fungi may lead to increased C stocks in those SOM fractions, they may ultimately lead to a slower accrual of *total* C stocks due to reductions in the rate of mineral associated organic matter (MAOM) formation. This slowing would be driven by declines in microbial CUE, biomass production, and stabilisation of necromass C to mineral exchange sites (Cotrufo *et al.*, 2013; Craig *et al.*, 2018). That said, this assumes that soils are composed of the mineral soil components suitable for stabilisation of organic C (e.g. clay-rich soils) and that mineral surfaces are not already saturated with organic C (Castellano *et al.*, 2015). Regardless, we argue that longer-term studies explicitly examining changes in specific SOM fractions are needed to assess the full magnitude of how the 'Gadgil effect' influences on C storage in forest soils.

Conclusions

The growing appreciation of mycorrhizal fungi as drivers of soil biogeochemical cycles has led to the promotion of incorporating tree mycorrhizal associations as trait integrators in modelling efforts (Phillips *et al.*, 2013; Averill *et al.*, 2014; Sulman *et al.*, 2017). While these efforts have been fruitful in advancing model predictions of C and nutrient cycles in terrestrial ecosystems, these classifications run the risk of obscuring the vast phylogenetic and functional diversity among EM fungi (Pellitier & Zak, 2018; Zak *et al.*, 2019) as well as top-down climatic controls on distribution (Read, 1991; Steidinger *et al.*, 2019), which are potentially key in understanding effects on ecosystem processes such as the 'Gadgil effect' (Fernandez & Kennedy, 2016). Given the notable differences that we observed between adjacent sites that differed in both plant and fungal community composition, we caution against generalising about the role of EM fungal suppression of SOM decomposition without further examination of both mechanism and context dependency. Instead, we advocate the implementation of high-throughput molecular approaches coupled with experiments across natural environmental gradients

as well as the use of techniques that specifically track resource movement from substrates into particular microbial guilds (Nuccio *et al.*, 2013), to improve our understanding of how EM-saprotroph interactions affect belowground forest C and N cycling.

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Author contributions

CWF and PGK conceived and designed the experiments. CWF led the field and laboratory work, data analysis and manuscript preparation. CRS helped with fieldwork and performed litter chemical analyses. CWF, CRS and PGK analysed the data and wrote the manuscript.

ORCID

Christopher W. Fernandez https://orcid.org/0000-0002-6310-6027
Craig R. See https://orcid.org/0000-0003-4154-8307

References

Amend AS, Seifert KA, Bruns TD. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Molecular Ecology* **19**: 5555–5565.

Anderson IC, Genney DR, Alexander IJ. 2014. Fine-scale diversity and distribution of ectomycorrhizal fungal mycelium in a Scots pine forest. *New Phytologist* **201**: 1423–1430.

Austin AT, Vivanco L, Gonzalez-Arzac A, Perez LI. 2014. There's no place like home? An exploration of the mechanisms behind plant litter–decomposer affinity in terrestrial ecosystems. *New Phytologist* **204**: 307–314.

Averill C, Hawkes CV. 2016. Ectomycorrhizal fungi slow soil carbon cycling. *Ecology Letters* **19**: 937–947.

Averill C, Turner BL, Finzi AC. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Bahram M, Põlme S, Kõljalg U, Zarre S, Tedersoo L. 2012. Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist* **193**: 465–473.

Baldrian P. 2017. Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology Reviews* **41**: 109–130.

Berg B. 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest ecology and Management* **133**: 13–22.

Berg B, Lindberg T. 1980. *Is litter decomposition retarded in the presence of mycorrhiza in forest soil? Internal report 95*. Uppsala, Sweden: Swedish Coniferous Forest Project, 10.

Bödeker IT, Clemmensen KE, Boer W, Martin F, Olson Å, Lindahl BD. 2014. Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist* **203**: 245–256.

Bödeker IT, Lindahl BD, Olson Å, Clemmensen KE. 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. *Functional Ecology* **30**: 1967–1978.

Brzostek ER, Dragoni D, Brown ZA, Phillips RP. 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. *New Phytologist* **206**: 1274–1282.

Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N. 2017. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology* **2**: 16242.

Castellano MJ, Mueller KE, Olk DC, Sawyer JE, Six J. 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change Biology* **21**: 3200–3209.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* **205**: 1525–1536.

Cornwell WK, Cornelissen JH, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindegu N *et al.* 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**: 1065–1071.

Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E. 2013. The Microbial Eficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* **19**: 988–995.

Courty PE, Pritsch K, Schloter M, Hartmann A, Garbaye J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytologist* **167**: 309–319.

Craig ME, Turner BL, Liang C, Clay K, Johnson DJ, Phillips RP. 2018. Tree mycorrhizal type predicts within-site variability in the storage and distribution of soil organic matter. *Global Change Biology* **24**: 3317–3330.

Dickie IA, Xu B, Koide RT. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156**: 527–535.

Edgar RC. 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*: 081257.

Fernandez CW, Kennedy PG. 2016. Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? *New Phytologist* **209**: 1382–1394.

Fernandez CW, Nguyen NH, Stefanski A, Han Y, Hobbie SE, Montgomery RA, Reich PB, Kennedy PG. 2017. Ectomycorrhizal fungal response to warming is linked to poor host performance at the boreal-temperate ecotone. *Global Change Biology* **23**: 1598–1609.

Finlay RD. 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany* **59**: 1115–1126.

Fisher FM, Gosz JR. 1986. Effects of trenching on soil processes and properties in a New Mexico mixed-conifer forest. *Biology and Fertility of Soils* **2**: 35–42.

Gadgil PD, Gadgil RL. 1975. Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. *New Zealand Journal of Forestry Science* **5**: 35–41.

Gadgil RL, Gadgil PD. 1971. Mycorrhiza and litter decomposition. *Nature* **233**: 133–133.

Genney DR, Anderson IC, Alexander IJ. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist* **170**: 381–390.

Gholz HL, Wedin DA, Smitherman SM, Harmon ME, Parton WJ. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology* **6**: 751–765.

Harmer R, Alexander IJ. 1985. Effects of root exclusion on nitrogen transformations and decomposition processes in spruce humus. In: Fitter AH, Atkinsson D, Read DJ, Usher MB, eds. *Ecological interactions in soils: plant, microbes and animals*. Oxford, UK: Blackwell, 269–277.

Hobbie EA, Diepen LT, Lilleskov EA, Ouimette AP, Finzi AC, Hofmockel KS. 2014. Fungal functioning in a pine forest: evidence from a ^{15}N-labeled global change experiment. *New Phytologist* **201**: 1431–1439.

Hobbie SE. 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. *Ecosystems* **8**: 644–56.

Hobbie SE. 2008. Nitrogen effects on decomposition: A five-year experiment in eight temperate sites. *Ecology* **89**: 2633–2644.

Ishida TA, Nara K, Hogetsu T. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. *New Phytologist* **174**: 430–440.

Kaye JP, Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution* **12**: 139–143.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A *et al.* 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics* **47**: 410–415.

Koide RT, Courty PE, Garbaye J. 2007. Research perspectives on functional diversity in ectomycorrhizal fungi. *New Phytologist* **174**: 240–243.

Koide RT, Fernandez CW, Malcolm G. 2014. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist* **201**: 433–439.

Koide RT, Wu T. 2003. Ectomycorrhizas and retarded decomposition in a *Pinus resinosa* plantation. *New Phytologist* **158**: 401–407.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Burns TD, Bengtsson-Palme J, Callaghan TM *et al.* 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Kuyper TW. 2017. Carbon and energy sources of mycorrhizal fungi: obligate symbionts or latent saprotrophs? In: Johnson NC, Gehring CA, Jansa J, eds. *Mycorrhizal Mediation of Soil*. Amsterdam, the Netherlands: Elsevier, 357–374.

Kyaschenko J, Clemmensen KE, Karltun E, Lindahl BD. 2017. Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecology Letters* **20**: 1546–1555.

Lilleskov EA, Bruns TD, Horton TR, Lee Taylor D, Grogan P. 2004. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. *FEMS Microbiology Ecology* **49**: 319–332.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* **173**: 611–620.

Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi–potential organic matter decomposers, yet not saprotrophs. *New Phytologist* **205**: 1443–1447.

Maaroufi NI, Nordin A, Palmqvist K, Hasselquist NJ, Forsmark B, Rosenstock NP, Wallander H, Gundale MJ. 2019. Anthropogenic nitrogen enrichment enhances soil carbon accumulation by impacting saprotrophs rather than ectomycorrhizal fungal activity. *Global Change Biology* **25**: 2900–2914.

Mayor JR, Henkel TW. 2006. Do ectomycorrhizas alter leaf-litter decomposition in monodominant tropical forests of Guyana? *New Phytologist* **169**: 579–588.

McGuire KL, Zak DR, Edwards IP, Blackwood CB, Upchurch R. 2010. Slowed decomposition is biotically mediated in an ectomycorrhizal, tropical rain forest. *Oecologia* **164**: 785–795.

Melillo JM, Aber JD, Muratore JF. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**: 621–626.

Midgley MG, Brzostek E, Phillips RP. 2015. Decay rates of leaf litters from arbuscular mycorrhizal trees are more sensitive to soil effects than litters from ectomycorrhizal trees. *Journal of Ecology* **103**: 1454–1463.

Nguyen NH, Smith D, Peay K, Kennedy P. 2015. Parsing ecological signal from noise in next generation amplicon sequencing. *New Phytologist* **205**: 1389–1393.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2018. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109.

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environmental Microbiology* **15**: 1870–1881.

Orwin KH, Kirschbaum MU, St. John MG, Dickie IA. 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecology Letters* **14**: 493–502.

Palmer JM, Jusino MA, Banik MT, Lindner DL. 2018. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ* **6**: e4925.

Pellitier PT, Zak DR. 2018. Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. *New Phytologist* **217**: 68–73.

Pena R, Tejedor J, Zeller B, Dannenmann M, Polle A. 2013. Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. *New Phytologist* **199**: 520–528.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist* **199**: 41–51.

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC. 2012. Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. *Ecology Letters* **15**: 1042–1049.

R Core Team. 2017. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. URL: https://www.R-project.org/Rognes

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytologist* **157**: 475–492.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Schlesinger WH, Bernhardt ES. 2013. *Biogeochemistry: an analysis of global change*. Waltham, MA, USA: Academic Press.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kogel-Knabner I, Lehmann J, Manning DAC *et al.* 2011. Persistence of soil organic matter as an ecosystem property. *Nature* **478**: 49–56.

Smith DP, Peay KG. 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLoS ONE* **9**: e90234.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. New York, NY, USA: Academic Press.

Smith GR, Wan J. 2019. Resource-ratio theory predicts mycorrhizal control of litter decomposition. *New Phytologist* **223**: 1595–1606.

Staaf H. 1988. Litter decomposition in beech forests—effects of excluding tree roots. *Biology and Fertility of Soils* **6**: 302–305.

Steidinger BS, Crowther TW, Liang J, Van Nuland ME, Werner GDA, Reich PB, Nabuurs GJ, de-Miguel S, Zhou M, Picard N *et al.* 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* **569**: 404.

Sterkenburg E, Bahr A, Brandström Durling M, Clemmensen KE, Lindahl BD. 2015. Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist* **207**: 1145–1158.

Sterkenburg E, Clemmensen KE, Ekblad A, Finlay RD, Lindahl BD. 2018. Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *ISME Journal* **12**: 2187.

Sulman BN, Brzostek ER, Medici C, Shevliakova E, Menge DN, Phillips RP. 2017. Feedbacks between plant N demand and rhizosphere priming depend on type of mycorrhizal association. *Ecology Letters* **20**: 1043–1053.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U. 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* **180**: 479–490.

Tedersoo L, Lindahl B. 2016. Fungal identification biases in microbiome projects. *Environmental Microbiology Reports.* **8**: 774–779.

Tedersoo L, Bahram M, Toots M, Diedhiou AG, Henkel TW, Kjøller R, Morris MH, Nara K, Nouhra E, Peay KG *et al.* 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**: 4160–4170.

Tilman D. 1982. *Resource competition and community structure*. Princeton, NJ, USA: Princeton University Press.

van der Wal A, Geydan TD, Kuyper TW, de Boer W. 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews* **37**: 477–494.

Vivanco L, Austin AT. 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. *Journal of Ecology* **96**: 727–736.

Walker JK, Cohen H, Higgins LM, Kennedy PG. 2014. Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytologist* **202**: 287–296.

Zak DR, Pellitier PT, Argiroff W, Castillo B, James TY, Nave LE, Averill C, Beidler KV, Bhatnagar J, Blesh J *et al.* 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. *New Phytologist* **223**: 33–39.

Zhu W, Ehrenfeld JG. 1996. The effects of mycorrhizal roots on litter decomposition, soil biota, and nutrients in a spodosolic soil. *Plant and Soil* **179**: 109–118.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Root in-growth rates.

Fig. S2 The relationship between ectomycorrhizal root in-growth and soil fungal guild abundances.

Fig. S3 Litter mass loss results from Experiment 1.

Fig. S4 Nitrogen, carbon and lignin concentrations of incubated litter.

Fig. S5 Carbon-to-nitrogen and lignin-to-nitrogen ratios of incubated litter.

Fig. S6 Fungal guild abundances colonising litter.

Fig. S7 Dominant fungal genera colonising incubated litter.

Table S1 Forest site tree host properties.

Table S2 Soil pH and nutrient availability.

Table S3 Fungal guild assignments for fungal genera.

Table S4 Mixed model effects tests for litter chemistry.

Table S5 Trenching effects on the relationships between litter carbon and lignin contents and nitrogen content.

Table S6 Mixed model results testing the effects of on litter guild abundance.

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