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Shifting Fungal Guild Abundances are Associated with Altered Temperate Forest Soil Carbon Stocks

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Abstract

Despite the importance of fungi to forest carbon (C) cycling and increasing calls to include microbial interactions in ecosystem models, how shifting fungal guild abundances impact soil C stocks remains poorly quantified, particularly in mineral soils where most C is stored. Additionally, a greater understanding of how fungal interguild interactions affect belowground litter decomposition is needed to more fully characterize soil C dynamics. To address these knowledge gaps, we conducted a multi-year soil trenching experiment in two temperate *Pinus strobus* stands in Minnesota, USA. We found that after two years, trenching increased ectomycorrhizal fungal relative abundance while decreasing saprotrophic fungal relative abundance (decreased ectomycorrhizal/saprotrophic ratio) and concurrently decreased soil C stocks by 10%. The decreased C stocks were primarily due to changes in particulate organic matter and were largely constrained to the top 5 cm of the soil. Trenching also stimulated both root and fungal litter decomposition in surface soils. Together, these results support the often proposed but rarely tested hypothesis that shifting fungal guild abundances promote soil C accumulation. However, they also suggest this effect may be most relevant for short-term C storage in upper soil layers.

Key words: Soil carbon stocks; Ectomycorrhizal Fungi; Gadgil effect; Mineral-associated organic matter; Particulate organic matter; Fungal necromass.

Highlights

- Trenching reduced ectomycorrhizal/saprotrophic fungal ratios and soil carbon stocks
- Surface-soil particulate organic matter was most reduced by trenching
- Trenching increased root litter and fungal necromass decomposition

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Introduction

Because soils are the largest active terrestrial reservoir of carbon (C), understanding the retention and release of C in and from this pool is critical for understanding the global C cycle and its response to anthropogenic change (Schlesinger 1999). In many temperate forests, the decomposition of soil organic matter (SOM) is strongly influenced by interactions between two functional groups, also called guilds, of fungi: saprotrophic and ectomycorrhizal fungi (EMF; Swift and others 1979). EMF are obligate plant symbionts, providing nutrients and water in exchange for plant-derived photosynthates (Smith and Read 2008). This supply of labile C allows EMF to enhance plant uptake of inorganic nitrogen (N; Khokon and others 2023) and certain EMF to mine N from organic sources such as fresh litter and/or SOM (Talbot and others 2013; Lindahl and Tunlid 2015; Dickie and others 2015; Kuyper 2017). In N-limited systems, which characterize most temperate forests (Jonard and others 2015; McLauchlan and others 2017), this N-mining by EMF exacerbates N-limitation of free-living saprotrophic fungi (hereafter saprotrophs), which rely on litter and SOM for both C and N. As a result of this fungal interguild competition, litter decomposition rates can be slowed (Averill and Hawkes 2016; Fernandez and others 2020), a phenomenon often referred to as the 'Gadgil effect' (Gadgil and Gadgil 1975). Despite increasing attention (Fernandez and others 2016; Frey 2019), key uncertainties remain regarding how fungal interguild interactions manifest outside of the litter layer in mineral soils where most soil C is stored (Jackson and others 2017).

To account for the diversity of SOM, especially with respect to its mean residence time (MRT), SOM has been operationally categorized into two major fractions: particulate organic matter (POM) and mineral-associated organic matter (MAOM; Kleber and others 2015). POM is formed through the fragmentation of fresh plant and microbial residues and is relatively accessible to decomposers, with an MRT of years to decades (Cotrufo and others 2015). MAOM typically comprises low-molecular weight compounds, formed through the microbial processing of POM, which are protected from further decomposition by chemical bonding with soil minerals, resulting in an MRT of centuries to millennia (Cotrufo and others 2015; Kleber and others 2015). As MAOM is primarily derived from the microbial processing of POM, the formation of both fractions depends on the activity of decomposers (Cotrufo and others 2013). Even with this link between POM degradation and MAOM formation, key factors differentiate their cycling. For example, MAOM formation requires mineral binding sites, whereas POM can accumulate without saturation (Castellano and others 2015). Although EMF have received some attention as sources of MAOM (Godbold and others 2006; Frey and others 2019) and possible destabilizers of MAOM (Jilling and others 2018; Cotrufo and others 2019; Wang and others 2020), how EMF–saprotroph interactions influence MAOM dynamics remains unclear. EMF–saprotroph interactions will likely have more pronounced effects on POM decomposition, as the decomposition of fresh litter is often limited by N availability (Moore and others 2015; Gill and others 2022). Given the difference in MRT between these fractions, the lack of explicit study of both POM and MAOM in the context of EMF–saprotroph interactions hinders understanding of the factors regulating long-term C storage in forest soils (Lavallee and others 2020).

To date, studies of the impacts of fungal interguild interactions on decomposition have largely focused on leaf litter in temperate forests (Gadgil and Gadgil 1975; Fernandez and others 2020; Lang and others 2021) and the organic horizons of boreal forests (Clemmensen and others 2013; Kyaschenko and others 2017; Clemmensen and others 2021). This is despite growing recognition that belowground C inputs, such as dead roots and fungal mycelium (that is, fungal necromass), can exceed leaf litter inputs to soils and are disproportionately important for long-term soil C storage (Clemmensen and others 2013; Jackson and others 2017; Huang and others 2020; Keller and others 2021). Additionally, studies comparing above- and belowground tissue decomposition have found that decomposition trends can differ both within and between tree species (Hobbie and others 2010; Freschet and others 2013). Further, the decomposition dynamics of fresh litters may not match those of bulk SOM and the factors that govern decomposition of a single litter type may not control soil C storage on an ecosystem scale (Schmidt and others 2011; Hobbie 2015). Therefore, if fungal interguild interactions affect belowground litter decomposition as they do aboveground decomposition, with EMF suppression being stronger for tissues with high C/N or that are rich in recalcitrant compounds that complex with N (for example, lignin in plants or melanin in fungi; Bull 1970; Averill and Hawkes 2016; Smith and Wan 2019; Fernandez and others 2020), ecosystem C retention is more likely to be impacted due to similar effects on decomposition of above and belowground litter types. Alternatively,

if fungal interguild interaction effects differ for decomposition of aboveground litter versus belowground tissues or SOM, any conclusions concerning soil C storage drawn from leaf litter studies may be misleading.

The focus on leaf litter in studies of fungal interguild interactions also overlooks heterogeneity of SOM with depth, which ranges from recently senesced tissues in the upper, organic-rich layers to further decomposed SOM deeper in the soil profile (Lindahl and others 2007). Throughout this continuum, key differences in substrate chemistry, microbial community composition, and edaphic factors likely influence effects of fungal guild interactions on decomposition (Carteron and others 2021). For example, fresh litter with high C/N in the upper organic soil may promote competitive suppression of saprotrophs by EMF through their N-mining (Jobbágy and Jackson 2000; Carteron and others 2021). Additionally, due to high organic matter inputs, the upper layers of the mineral soil have elevated abundance and spatial overlap of saprotrophs and EMF, increasing the possibility of interaction (Lindahl and others 2007; Maaroufi and others 2019; Carteron and others 2021). In the deeper soil horizons, with lower SOM content and C/N ratio, EMF typically dominate, with comparatively few saprotrophs (higher EMF/saprotroph ratio), potentially leading to less interguild competition (Lindahl and others 2007; Kyaschenko and others 2017). Further, saprotrophs at lower depths are more energy- and C-limited, rather than N-limited (Fontaine and others 2007). This lack of spatial overlap between EMF and saprotrophs, the higher N content, and energy limitation of saprotrophs with depth combine to suggest removing EMF will have less pronounced effects deeper in the soil profile. While these depth-explicit interguild dynamics have received study in the organic soil horizons of boreal forests, their importance in the mineral soil of temperate systems is less clear (Carteron and others 2021). Yet, given that most soil C is stored in the mineral soil, especially in temperate systems lacking well-developed organic horizons, such depth dependencies deserve more study (Jackson and others 2017).

To address the uncertainties regarding the impacts of fungal interguild interactions on soil C dynamics, we conducted a multi-year soil trenching experiment in two temperate *Pinus strobus* stands in Minnesota, USA. We hypothesized that decreasing the ratio of EMF to saprotrophs via trenching would decrease soil C stocks, due to enhanced decomposition of POM, with MAOM either increasing or being unaffected. Further, we hypothesized that effects of trenching on soil C stocks would decrease with depth, due to the lower abundance of saprotrophs (higher EMF/saprotroph ratio) in deeper soils. Finally, we also hypothesized that trenching would enhance the decomposition of belowground tissues, and that the magnitude of this effect would be stronger in tissues with higher C/N ratio and concentrations of recalcitrant compounds, like melanin, enhancing N-limitation.

Materials and Methods

Site Description

This experiment was conducted at the University of Minnesota Cedar Creek Ecosystem Science Reserve in central Minnesota, USA, a National Science Foundation Long Term Ecological Research site (45.42577N, 093.20852W). Specifically, this study was conducted in two second-growth *Pinus strobus* (eastern white pine) stands located 1.2 km apart, hereafter referred to as sites 1 and 2. The understories of both sites are sparse and do not contain many plants associated with ericoid mycorrhizae, which have also been implicated in interguild interactions (for example, Ward and others 2021). Suppression of *Pinus strobus* needle litter decomposition by EMF in the litter layer has been observed previously at site 1 (Fernandez and others 2020). Soils at both sites are poorly developed, very sandy (~ 89%) Udipsamments and lack an organic horizon (Grigal and others 1974). Eight replicate blocks were established at each of these sites in June 2018. Each block consisted of two side-by-side plots (1 m^2 each), one of which had root and EMF connections severed by passing a handsaw down to 30 cm from the top of the mineral soil every two weeks of the full growing season (hereafter trenching). The other plot was left untrenched as a control. Trenching has been the most common method used to study EMF–saprotroph interactions in relation to decomposition (as reviewed in Fernandez and Kennedy 2016). However, in severing root connections, trenching introduces labile root litter and fungal necromass C and N inputs that may prime litter and SOM decomposition (Dijkstra and others 2013). Addressing this issue experimentally, Gadgil and Gadgil (1975) incorporated root removal factorially with trenching and found no effect of root removal on the trenching effect on litter decomposition. Fisher and Gosz (1986) also found no evidence that these trenching-associated inputs impacted litter or SOM decomposition. Based on these findings, we believe that regular trenching represents a reasonable approach to

assessing the effect of EMF–saprotroph interactions on litter decomposition, especially over a period of multiple years, which allows any labile inputs to be expended (Fernandez and Kennedy 2016).

Soil Sampling and SOM Fractionation

To assess how trenching impacted soil C, soils were sampled at three different depths (0–5 cm, 5–10 cm, 10–20 cm from the litter-mineral soil interface) in August 2020 one week after regular trenching. After removing the litter layer, two replicate soil cores were collected from each plot using a 5.2 cm diameter split hammer corer, divided into depth increments, homogenized, and transported on ice to the lab for further processing. Soils were then sieved to 2 mm to isolate standing root stock and air-dried. Bulk density of each depth increment was calculated by taking cores in a 5 × 4 grid (20 total cores) spanning each site to capture spatial variation. Bulk density sampling was conducted outside of plots to minimize damage to plots, as it was assumed that trenching would not affect bulk density in these coarse soils.

To assess the effects of trenching on distinct SOM pools, we used a density- and size-fractionation procedure to separate POM and MAOM (Soong and Cotrufo 2015; Lavallee and others 2020). Briefly, light POM was less dense than 1.85 g cm^{-3} (as determined in sodium metatungstate); heavy POM was denser than 1.85 g cm^{-3} and larger than 53 μm (by wet sieving); and MAOM was denser than 1.85 g cm^{-3} and smaller than 53 μm. Detailed fractionation methods are provided in the supplementary materials. Each fraction (as well as bulk soil) was analyzed for C and N content by dry combustion (Costech 4010 Elemental Analyzer, Valencia, CA, USA). For simplicity of analyses, light and heavy POM fractions were summed to yield a single POM fraction (Lavallee and others 2020). To account for the differing sampling depth increments (0–5, 5–10, 10–20 cm) when analyzing across depths, C stocks are expressed on a volumetric basis (kg C m^{-3}). When pooled across the full 20 cm sampling depth, however, a more standard area-basis stock is used (kg C m^{-2}).

Fungal Guild Abundances

To assess the effects of trenching on fungal guild abundances, aliquots were sterilely sampled from soil cores at each depth described above, transported to the laboratory on ice, and frozen at −80 °C before extraction. DNA was extracted from 250 mg soil samples using Qiagen DNEasy Power Soil Pro kits. Soil fungal communities were analyzed using high throughput sequencing (HTS), as in Maillard and others (2023). We present analysis on the guild level (that is, ectomycorrhizal and saprotrophic) based on sums of rarefied sequence read counts from those guilds. These two guilds dominated our samples with EMF and saprotrophic fungi accounting for 46.9% and 33.4% of total fungal read counts, respectively. To quantify total fungal abundances in the same samples, we used real-time quantitative PCR (qPCR), expressed as $\log_{10}$ of numbers of fungal 18S copies per gram of dry soil. More detailed descriptions of molecular methods, including guild categorization, are provided in the supplementary materials.

Belowground Tissue Decomposition

To assess the effects of trenching on belowground tissue decomposition, roots and fungal mycelia (hereafter necromass) were incubated in mesh bags (initial mass 150 mg in 53 μm-mesh bags) in mineral soil at a depth of 2.5 cm from the litter-mineral soil interface in trenched and control plots. Root litter and fungal necromass types are described in Table 1. Fine root (< 2 mm in diameter) litter of four tree species was collected and pooled from stands within 1 km of study sites, then dried at 60°C for 3 days before being deployed in monospecific bags. Root litter species were selected to represent locally common tree species with differing tissue chemistry (for example, C/N), phylogeny (gymnosperm vs. angiosperm), and mycorrhizal association (arbuscular vs. EMF), which have previously been implicated in determining root decomposition dynamics (See and others 2019; Table 1). Root litter bags were incubated for 12 months (August 2019–August 2020). Fungal necromass of two species was grown in liquid culture (1/2 concentration potato dextrose broth) for 30 days for *Mortierella elongata*, and 60 days for *Meliniomyces bicolor* to achieve similar masses. After sufficient mass was generated, cultures were rinsed with DI water and dried at 26°C before being ground with a mortar and pestle and passed through a 250 μm sieve to homogenize them. Fungal necromass was incubated in the field for 3 months (August–October 2019). Necromass species were selected to vary in C and N content, as well as melanin content, which has been shown to be a major control over necromass decomposition (Fernandez and others 2018; See and others 2021; Table 1), and both species are naturally present in these sites (data not shown). Initial C and N concentrations of roots and necromass were determined by dry combustion. Both root and necromass species are referred to by genus name.

Table 1. Belowground Litter Types with Initial Chemistry

Species	%C	%N	C/N Ratio	Phylogeny	Mycorrhizal association
Root litter					
Thuja occidentalis	49.96 ± 1.89	0.71 ± 0.02	60.68 ± 3.61	Gymnosperm	Arbuscular
Acer rubrum	42.74 ± 3.49	1.16 ± 0.08	31.73 ± 2.63	Angiosperm	Arbuscular
Quercus ellipsoidalis	44.24 ± 2.52	0.92 ± 0.12	41.51 ± 3.38	Angiosperm	Ectomycorrhizal
Pinus strobus	40.78 ± 2.83	0.86 ± 0.09	40.88 ± 4.10	Gymnosperm	Ectomycorrhizal

Species	%C	%N	C/N Ratio	Guild	Melanin Content
Fungal Necromass					
Meliniomyces bicolor	47.98 ± 2.88	1.99 ± 0.04	20.63 ± 1.01	Ectomycorrhizal	High
Mortierella elongata	47.63 ± 4.67	3.08 ± 0.33	13.44 ± 2.63	Saprotroph	Low

Mean values are presented ± one standard deviation (n = 3).

Statistical Analyses

To understand the effects of trenching and depth on SOM fraction stocks and fungal guild abundances, we conducted ANOVA (car package in R; Fox and Weisberg 2019) on linear mixed-effects models (nlme package in R; Pinheiro and others 2021) with block nested within site as random effects. This nesting scheme was used to control for block-level differences in unmeasured metrics such as ground cover plant biomass as well as to account for site differences. Response variables were natural-log transformed, whenever appropriate, to meet assumptions of normality of residuals, based on visual inspection of QQ-plots. Interactions between predictor variables were tested and removed if not significant. Significance was defined as $p < 0.05$ and was calculated based on Wald chi-square tests, which are preferred over standard F tests for mixed linear effects models fit in the nlme package (Fox and Weisberg 2019; Pinheiro and others 2021). Pairwise differences between treatments and/or depths were determined using post-hoc comparisons with Holm-corrected p-values (Holm 1979). To understand the effects of trenching on root litter and necromass decomposition, we used the same model structure (but without depth as a predictor) with proportion of initial mass remaining as the response variable. To explore links between the EMF/saprotroph ratio and soil C stocks, these variables were regressed against one another using the mixed-model structure described above with depth and trenching as predictors. The effect sizes of trenching on each of these variables were also calculated as natural-log response ratios (hereafter log response ratios) and regressed against one another, with only site as a random effect. Log response ratios (LRR) were calculated as $LRR = \ln(X_T/X_C)$ where X_T denotes the value of a given response variable in the trenched plot and X_C denotes the value of that variable in the control plot. A log response ratio was calculated for each block (that is, one pair of trenched-control plots). To assess total fungal abundance on soil C stocks and their response to trenching, total fungal abundance (qPCR) was regressed against soil C stocks and their log response ratios were regressed against one another.

Results

Root Stocks and Fungal Abundances

Both total and fine (not shown) root standing stocks decreased with depth ($\chi^2_2 = 7.55$; $p = 0.023$; Figure 1a). Trenching reduced total root stocks by 42.6% on average across depths ($\chi^2_1 = 26.08$; $p < 0.001$; Figure 1a). Overall, the EMF/saprotroph ratio was reduced by 36% with trenching ($\chi^2_1 = 7.44$; $p = 0.006$) and decreased with depth ($\chi^2_2 = 14.93$; $p < 0.001$); however, the effect of trenching did not vary with depth (treatment/depth $\chi^2_2 = 3.30$; $p = 0.192$; Figure 1b). Similarly, EMF relative abundances were significantly reduced by trenching (18.8%; $\chi^2_1 = 9.82$; $p = 0.002$) but did not vary significantly across depths ($\chi^2_2 = 2.84$; $p = 0.242$). Conversely, saprotrophic fungal relative abundances increased significantly with trenching ($\chi^2_1 = 3.91$; $p = 0.041$) and depth ($\chi^2_2 = 31.4$; $p < 0.001$). Across all samples, EMF and saprotrophic fungal relative abundances were negatively correlated ($\chi^2_1 = 273.32$; $p < 0.001$; Marginal $R^2 = 0.728$), with neither treatment nor depth altering this relationship. Total fungal abundance was highest in the top 5 cm of soil ($\chi^2_2 = 19.04$; $p < 0.001$) and was reduced by 33.4% (in the non-$\log_{10}$ scale), on average, by trenching ($\chi^2_1 = 10.59$; $p = 0.001$), an effect that

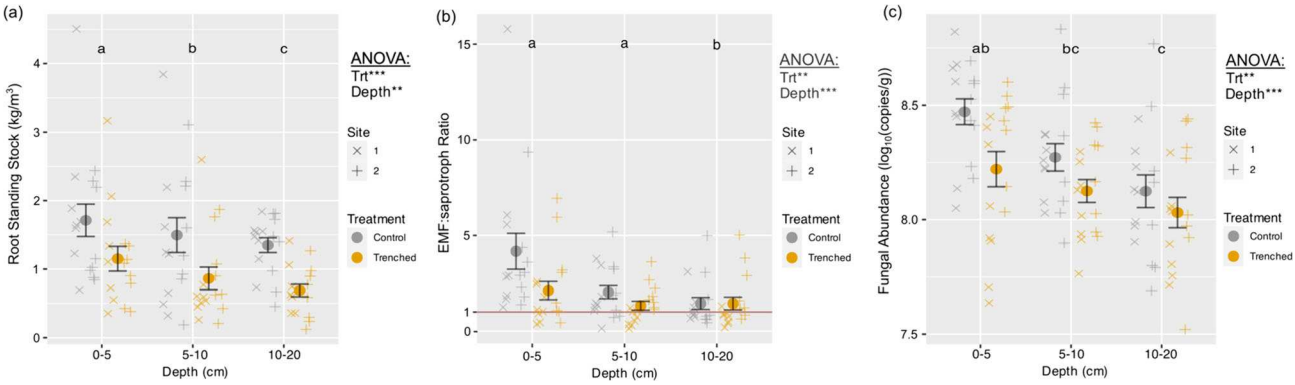

Figure 1. Trenching effects on **a** root standing stock and **b** EMF/saprotrophic fungal ratio, and **c** total fungal abundance (based on qPCR). Red line in **b** is at EMF/saprotroph ratio = 1. For all panels, solid, round points indicate mean values for each treatment (trt), with color denoting control or trenched plot. Individual plot values are shown by site (site denoted by shape). Letters above indicate statistically significant differences ($p < 0.05$) between depths according to pairwise comparisons with Holm-corrected p-values. For panel (**b**) all significant linear mixed-model ANOVA results are provided, with *** denoting $p < 0.001$, ** denoting $p < 0.01$, and * denoting $p < 0.05$.

did not vary significantly with depth (treatment/depth $\chi^2_2 = 1.68$; $p = 0.433$; Figure 1c). Total fungal abundance was also positively correlated with the EMF/saprotroph ratio ($\chi^2_1 = 35.5$; $p < 0.001$; marginal $R^2 = 0.39$).

Soil C stocks and Fractions

Bulk soil C was dominated by POM (65.4% of bulk soil C in control plots, on average; Figure 2b), and, as such, bulk soil C trends were primarily driven by POM. Both bulk soil C and POM C stocks declined ~ twofold with each depth increment (Bulk $\chi^2_2 = 158.16$; POM $\chi^2_2 = 216.4$; both $p < 0.001$), while MAOM C stocks only differed between the top and bottom 10 cm increments ($\chi^2_2 = 22.42$; $p < 0.001$; Figure 2). Across depths, and in support of our hypothesis, trenching significantly reduced the bulk soil (volumetric) C stocks from 9.76 ± 0.59 kg C m^{-3} to 8.78 ± 0.41 kg C m^{-3}, an average decrease of 10% after two years ($\chi^2_1 = 4.54$; $p = 0.033$; Figure 2a). Also as hypothesized, this was driven by the trenching-induced reduction in POM (12.4%; $\chi^2_1 = 6.56$; $p = 0.010$; Figure 2c), rather than the relatively unchanged MAOM (2.3% decrease; $\chi^2_1 = 0.418$; $p = 0.518$; Figure 2d). Further, while the strength of trenching effects on bulk soil C stocks did not vary significantly with depth (treatment/depth $\chi^2_2 = 1.01$; $p = 0.605$), the effect of trenching on the POM C stock was strongest (17.5% decrease) in the top 5 cm of soil (treatment/depth $\chi^2_2 = 4.7$; $p = 0.030$; Figure 2c). When C stocks were pooled across the full 20 cm sampling depth, trenching reduced soil C stocks from 1.81 ± 0.1 kg C m^{-2} to 1.65 ± 0.08 kg C m^{-2}, a relatively weak decrease of 8.6% ($\chi^2_1 = 3.31$; $p = 0.069$; Figure 2b). The significant effect of trenching on POM C stocks ($\chi^2_1 = 4.04$; $p = 0.044$; Figure 2b) and lack of significant effect on MAOM C stocks, however, remained ($\chi^2_1 = 0.38$; $p = 0.538$; Figure 2b). Additionally, trenching did not have any effect on the C/N ratios of the bulk soil, POM, or MAOM, though they all increased significantly with depth (all $p < 0.001$).

Linking Changes in Soil C Stocks with Changes in Fungal Guild Abundances

The effect of trenching (as log response ratio) on bulk soil C stocks was significantly correlated with the effect of trenching (as log response ratio) on the EMF/saprotroph ratio ($\chi^2_1 = 4.23$; $p = 0.039$; $R^2 = 0.083$; Figure 3a). Volumetric bulk soil C stocks (not log response ratios to trenching) were also significantly positively correlated with EMF/saprotroph ratios ($\chi^2_1 = 7.52$; $p = 0.006$; marginal $R^2 = 0.500$; Figure 3b). This relationship did not vary with depth or treatment. Further, when the EMF/saprotroph ratio was included as a covariate, trenching no longer significantly affected C stocks ($\chi^2_1 = 1.92$; $p = 0.165$), regardless of depth. This, like the effect size regression, indicates that trenching primarily impacted C stocks by changing EMF/saprotroph ratios. Total fungal abundance was also positively correlated with C stocks across depths and treatments ($\chi^2_1 = 7.31$; $p = 0.007$, marginal $R^2 = 0.502$) and also rendered the trenching variable no longer significant. However, the log response ratio of total fungal abundance to

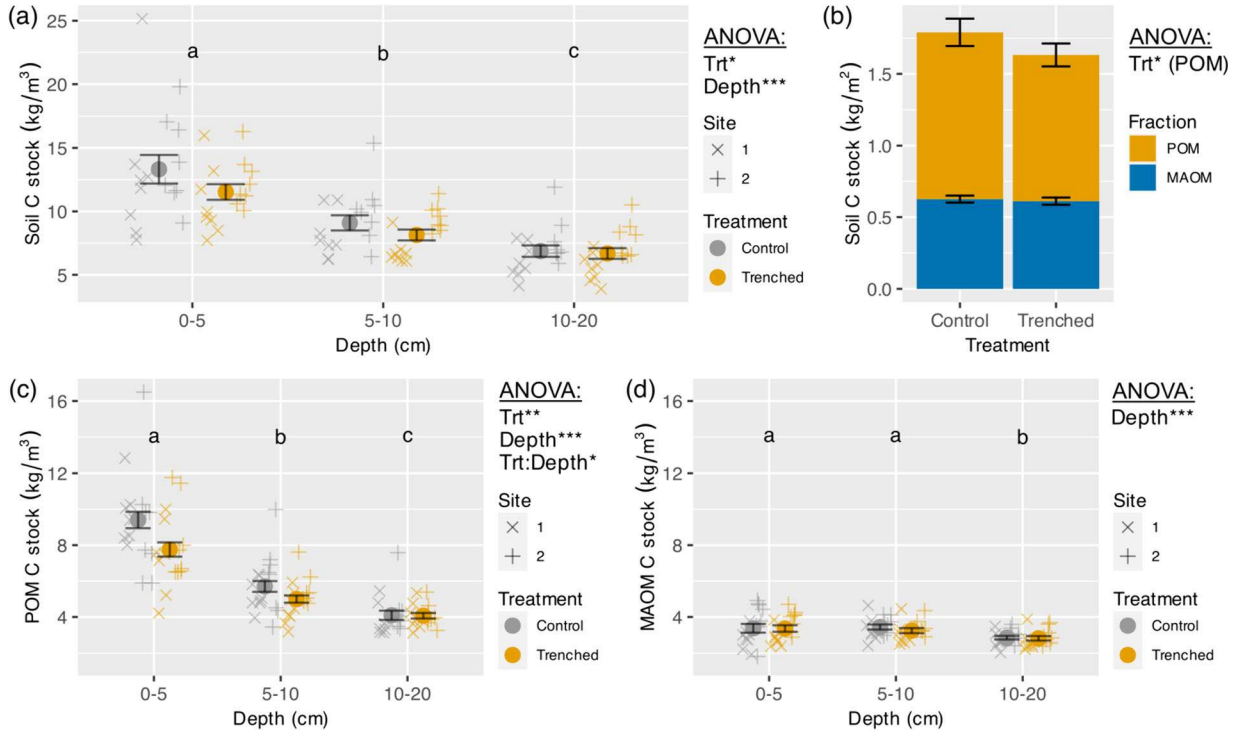

Figure 2. Treatment effects on soil carbon (C) stocks: **a** bulk soil volumetric C stock (kg/m^3) by depth and treatment, **b** bulk soil areal C stock (kg/m^2) by treatment (trt), split into different fractions, **c** particulate organic matter (POM) volumetric C stock (kg/m^3) by depth and treatment, and **d** mineral-associated organic matter (MAOM) volumetric C stock (kg/m^3) by depth and treatment. All error bars represent one standard error. For panels **a**, **c**, and **d** solid, round points indicate mean values for each treatment, with color denoting control or trenched plot. Individual plot values are shown by site (site denoted by shape). Letters above indicate statistically significant differences ($p < 0.05$) between depths according to pairwise comparisons with holm-corrected p-values. For panel (**b**) all significant linear mixed-model ANOVA results are provided, with *** denoting $p < 0.001$, ** denoting $p < 0.01$, and * denoting $p < 0.05$.

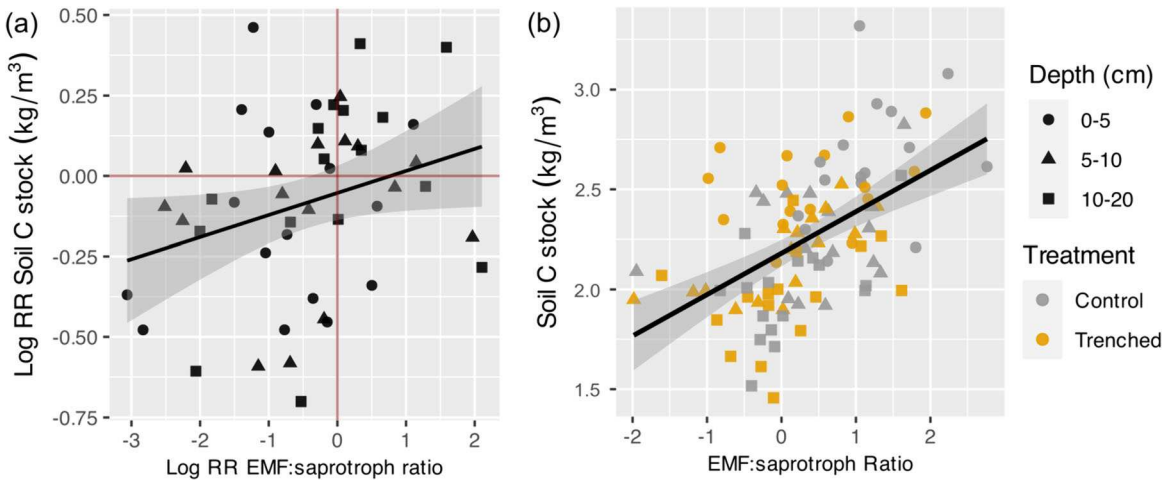

Figure 3. Correlations between EMF/saprotroph ratio and soil C stocks. Panel **a** represents the response of C stocks to trenching (as natural-log response ratios). Panel **b** represents regressions of EMF/saprotroph ratio and soil C stocks with both axes natural-log transformed. Symbol shape indicates depth for both panels and color indicates treatment in panel (**b**).

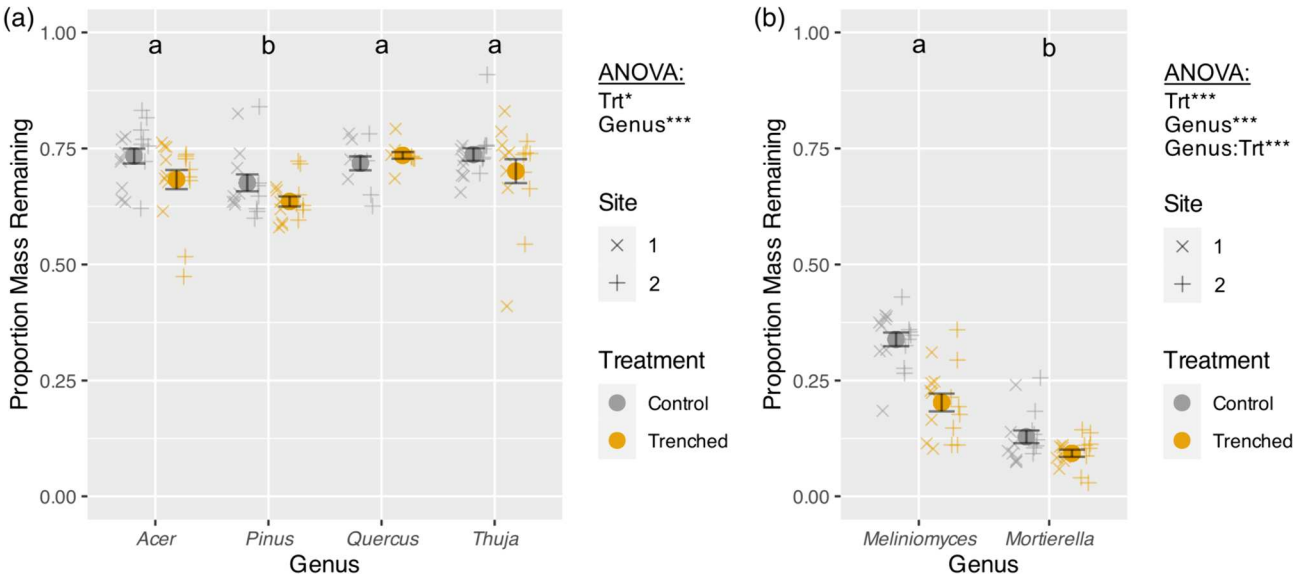

Figure 4. Proportion mass remaining of **a** fine root litter after 12 months of decomposition and **b** fungal necromass after 3 months of decomposition by genus. Solid, round points indicate mean values for each treatment (trt), with color denoting control or trenched plot. Error bars represent one standard error. Individual plot values are shown by site (site denoted by shape). Letters above indicate statistically significant differences ($p < 0.05$) between genera according to pairwise comparisons with Holm-corrected p-values. Linear mixed-model ANOVA results are provided, with *** denoting $p < 0.001$, ** denoting $p < 0.01$, and * denoting $p < 0.05$.

trenching was only marginally correlated with the log response ratio of the C stock to trenching ($\chi^2_1 = 2.92$; $p = 0.087$; marginal $R^2 = 0.058$).

Belowground Tissue Decomposition

As hypothesized, trenching significantly stimulated root litter decomposition ($\chi^2_1 = 6.37$; $p = 0.012$; Figure 4a), by an average of 4.5%. Response to trenching did not differ by root species (treatment/species $\chi^2_3 = 2.67$; $p = 0.445$; Figure 4a). Fungal necromass decomposition was also significantly stimulated by trenching, by an average of 36.5% ($\chi^2_1 = 35.63$; $p < 0.001$); however, this effect varied by species (treatment/species $\chi^2_1 = 12.18$; $p < 0.001$; Figure 4b). Specifically, trenching increased decomposition more for the higher C/N and more melanized *Meliniomyces* (40%) than for *Mortierella* (27.4%) necromass.

Discussion

After two years, we found that trenching decreased soil C stocks in the top 20 cm by approximately 10%, consistent with our hypothesis. This decrease in C stock was strongest in the top 5 cm of soil and was driven by changes to the short-MRT POM pool, while long-MRT MAOM remained unaffected. As hypothesized, C stocks were positively correlated with EMF/saprotroph ratios and the observed decrease to C stocks with trenching were significantly associated with decreases in the EMF/saprotroph ratio. Specifically, decreases in C stocks were associated with decreases in EMF relative abundance and increases in saprotrophic relative abundance. The stock results were corroborated by increased decomposition of belowground litters in the top 5 cm of soil with trenching. While these results collectively support the importance of fungal interguild interactions for soil C storage, they also suggest caution in extrapolation to deeper soil C, which remained unchanged with trenching even at a relatively shallow depth of 10–20 cm.

Trenching Decreased Soil C and POM Stocks

While positive effects of EMF on soil C stocks have been previously suggested in global analyses (Averill and others 2014), this was inferred from C/N ratios, a trend later shown to be driven by EMF reducing N stocks, rather than increasing C stocks, as originally implied (Zhu and others 2018). Our data, however, indicate that antagonistic EMF–saprotroph interactions can increase soil C stocks in temperate coniferous forests. While these dynamics

have been most frequently studied in bulk C in organic horizons of boreal systems (Kyaschenko and others 2017; Sterkenberg and others 2018; Maaroufi and others 2019), trenching was also shown to decrease depth-pooled soil C stocks by 31% over 10 years in mineral soil of a hardwood-dominated temperate forest (Whalen and others 2021). Taken together, these different datasets support the idea that EMF, either alone and/or through their interactions with saprotrophs, can promote C storage across systems with different soil types (for example, temperate mineral soil or boreal organic horizons) and tree lineages (conifer or hardwood).

Higher soil C stocks in control plots, driven by an increase of POM is in line with our hypothesis, as reduced saprotrophic decomposition activities in the presence of EMF should lead to less processing of POM into MAOM and CO_2 (Cotrufo and others 2013). They are also consistent with the observed decreases in root litter and fungal necromass decomposition (both components of POM; Lavallee and others 2020) as well as with results from boreal organic horizons, which are exclusively POM (Kyaschenko and others 2017; Sterkenberg and others 2018; Maaroufi and others 2019). Further, our results align with a previous mesocosm and modeling study that found that EMF increased POM pools, leaving MAOM unchanged (Moore and others 2015). While we did find that the trenching effect was strongest in the top 5 cm of soil for POM C stocks, significant depth-dependency was not observed in bulk soil C stocks or EMF/saprotroph ratios. Lack of depth-dependent trenching effects on bulk soil C is contrary to our hypotheses as well as results from boreal horizons (Kyaschenko and others 2017), and occurred despite a consistent, though insignificant, trend in depth effects in EMF/saprotroph ratios and soil C stocks. We suspect the lack of depth dependency may be due to the shallow, poorly developed soil profile at our study sites (Grigal and others 1974). Nonetheless, the consistent, albeit weak change in trenching effects with depth indicates that the depth-specific effects of interguild interactions warrant further study in more developed profiles.

The two-year duration of our study may have been insufficient to capture any influence of EMF on the dynamics of the slowly cycling MAOM pool (Lavallee and others 2020). This possibility is suggested by a long-term root removal experiment, which found that 20 years of trenching reduced POM but had a stronger, positive effect on MAOM, leaving overall stocks unchanged (Pierson and others 2021). Further, given that the soils at our site are very sandy, it is also possible that all active mineral sites were already saturated (Cotrufo and others 2019). However, as MAOM standing stock is the result of both stabilization and destabilization processes, it is possible that the observed lack of change in MAOM does not suggest a lack of effect, but rather that fungal interguild interactions affect both inputs and outputs from this pool, though it is not possible to test this in the present study. Further tests in more mineral-rich systems are needed to fully understand the effects of EMF on MAOM. Nevertheless, our results suggest that fungal guild interactions can be important for soil C storage in POM-dominated systems and likely for short-term C and N cycling processes, such as soil fertility, elsewhere.

In addition to trenching reducing EMF inhibition of saprotrophic organic matter decomposition (decomposition pathway), another non-mutually exclusive mechanism that may explain the altered C stocks is that trenching reduced root and EMF inputs to the soil C pool (biomass pathway). The decomposition pathway is frequently invoked during explicit tests of the Gadgil effect, wherein EMF suppression of leaf litter decomposition is extrapolated to soil or ecosystem C storage (for example, Averill and others 2014). This has been supported in boreal systems (Kyaschenko and others 2017) and by results from an EMF abundance gradient, wherein EMF were positively related to mineral soil C concentrations (Craig and others 2018). On the other hand, the biomass pathway is suggested by studies stressing the importance of root and hyphal inputs to soil C (Godbold and others 2006; Huang and others 2020; Whalen and others 2021). Disentangling these two mechanisms in our study is challenging because relative abundances of both guilds (as well as total fungal abundance based on qPCR) were predictors of soil C stocks and were themselves negatively related to one another, raising the possibility that the relationship of relative abundance of any one guild with soil C stocks was spurious. While we avoid this issue here by focusing on the changes in the EMF/saprotroph ratio rather than assessing each guild independently, further analyses are needed to trace C inputs from litter versus EMF biomass into different SOM fractions to specifically elucidate the relative importance of the decomposition and biomass pathways for fungal interguild interactions on soil C storage.

Trenching Inhibits Decomposition of Belowground Tissues

We observed significant suppression of decomposition with an increased EMF/saprotroph ratio across both root and fungal necromass tissues. This is in line with previous results on root and fungal necromass decomposition in temperate mineral soils (Beidler and others 2021) as well as on necromass decomposition in boreal soils (Maillard and others 2021). Fungal necromass decomposed faster than roots (Hobbie and others 2010; See and others 2019), even given a four-fold shorter incubation time, matching previous analyses of these residues (Beidler and others 2020; Maillard and others 2021). The variation in decomposition response to trenching within and among tissue types was only partially consistent with our hypothesis. We expected that EMF would inhibit the decomposition of substrates with high C/N ratio and high abundance of recalcitrant, N-complexing compounds (for example, melanin or lignin) by mining substrates for N and outcompeting saprotrophs (Orwin and others 2011; Smith and Wan 2019; Fernandez and others 2020). However, trenching stimulated the decomposition of lower-C/N fungal necromass more strongly than the decomposition of relatively higher C/N roots. Further, the decomposition of roots of different species showed no significant variation in trenching response despite a range of C/N ratios. The response of fungal necromass to trenching did match the N-mining hypothesis, in terms of either C/N or melanin content; however, as only two necromass species were analyzed, we cannot disentangle which aspect of necromass chemistry contributed most to variation in trenching response. Collectively, our results suggest that while EMF can reduce saprotrophic decomposition of critical belowground C inputs to soils, it appears some other aspect of substrate chemistry controls the variation in decomposition response to trenching across tissue types.

Future Research Directions

Trenching-based experiments, as deployed here and commonly in other studies, cannot distinguish the effects of EMF from those of their host roots (Moore and others 2015). It is possible that observed Gadgil effects are primarily due to root competition/inputs and the assumed importance of EMF is a methodological artifact. Therefore, it is critical for further studies to isolate these effects, as well as the distinct C contributions from leaf litter, roots, and fungi to bulk SOM, MAOM, and POM. Also, while we did observe some trends in interguild interactions with depth over a small vertical sampling increment, the profile at our site is very shallow (Grigal and others 1974). In deeper, more developed profiles it is likely that EMF will exert impacts over a larger vertical scale, potentially leading to higher magnitude changes to C storage (Fernandez and Kennedy 2016). Finally, this study was designed to understand if EMF suppression of saprotrophic decomposition occurs belowground, and how this may translate to soil C storage and persistence. Therefore, we selected sites in which EMF suppression of leaf litter decomposition had previously been observed (Fernandez and others 2020). It is important to note that other studies have observed neutral or positive effects of fungal interguild interactions on leaf litter or organic horizon decomposition (Brzotek and others 2015; Lang and others 2021; Clemmensen and others 2021; Mayer and others 2023; Shao and others 2023). Because the correspondence between litter decomposition and mineral soil C trends has not yet been studied in these systems, the applicability of our results to other forests is unclear.

Conclusions

Our results extend previous studies of EMF–saprotroph interactions in leaf litter and organic horizons into the mineral soil, where the majority of C is stored (Jobbágy and Jackson 2000). In line with previous predictions, we found that decreases in the EMF/saprotroph ratio were linked to decreases in soil C stocks in temperate forests. This relationship was strongest in shallow soils and was more important for faster cycling C pools (POM), which have higher relevance for fertility and primary production than for slow-turnover C pools (MAOM). Given the importance of the soil C pool to the global C cycle and climate system, our findings that fungal interguild interactions influence mineral soil C stocks lend support to calls for the inclusion of fungal dynamics as well as edaphic context (that is, soil depth and SOM fraction) into ecosystem models used to predict C dynamics under anthropogenic global change (Schmidt and others 2011; Weider and others 2013; Sulman and others 2019; Shao and others 2023).

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DATA AVAILABILITY

Data and code associated with this manuscript are archived and available to editors and reviewers through the Environmental Data Initiative (EDI) at https://doi.org/10.6073/pasta/b7dc9bd0e0d4ed96b10672c8eb350ec8. Data and code will be made publicly available upon publication of the manuscript.

Declarations

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

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