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Differences in soil organic matter between EcM- and AM-dominated forests depend on tree and fungal identity

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Handling Editor: Joseph B. Yavitt

Open Research Statement:
Data (Hicks Pries et al. 2022) are available from the Environmental System Science Data Infrastructure for a Virtual Ecosystem (ESS-DIVE) repository at https://doi.org/10.15485/1894515. Code (Hicks Pries 2022) is available on Figshare at https://doi.org/10.6084/m9.figshare.19624254.v1.

Abstract

As global change shifts the species composition of forests, we need to understand which species characteristics affect soil organic matter cycling to predict future soil carbon (C) storage. Recently, whether a tree species forms a symbiosis with arbuscular (AM) versus ectomycorrhizal (EcM) fungi has been suggested as a strong predictor of soil carbon storage, but there is wide variability within EcM systems. In this study, we investigated how mycorrhizal associations and the species composition of canopy trees and mycorrhizal fungi relate to the proportion of soil C and nitrogen (N) in mineral-associations and soil C:N across four sites representing distinct climates and tree communities in the Eastern U.S. broadleaf forest biome. In two of our sites, we found the expected relationship of declining mineral-associated C and N and increasing soil C:N ratios as the basal area of EcM-associating trees increased. However, across all sites these soil properties strongly correlated with canopy tree and fungal species composition. Sites where the expected pattern with EcM basal area was observed were 1) dominated by trees with lower quality litter in the Pinaceae and Fagaceae families and 2) dominated by EcM fungi with medium distance exploration type hyphae, melanized tissues, and the potential to produce peroxidases. This observational study demonstrates that differences in soil organic matter between AM andEcM systems are dependent on the taxa of trees and EcM fungi involved. Important information is lost when the rich mycorrhizal symbiosis is reduced to two categories.

Keywords: arbuscular mycorrhizae, C:N, ectomycorrhizae, Fagaceae, fungi, MAOM, mineral-associated organic matter, Pinaceae, soil carbon, soil nitrogen, species composition, tree community

Introduction

Forest soils store 75% of global soil carbon (C; Jackson et al. 2017), and managing forests for C storage has some of the highest climate change mitigation potential of natural climate solutions (Griscom et al. 2017). Our ability to manage forests for soil C storage is challenged by the changing composition of forests due to climate change, pests and pathogens, and shifting fire regimes (Nowacki and Abrams 2008, Morin and Liebhold 2015, Jo et al. 2019). In order to predict the future potential and reliability of forest soil C storage, we need a better understanding of how tree species affect the quality and quantity of soil organic matter (SOM).

One key way that tree species affect SOM is through their association with mycorrhizal fungi. Although mycorrhizal fungi are biotrophs—they provide plants with soil nutrients in return for photosynthate—they interact with OM and free-living soil saprotrophs, making them critical mediators of OM formation and retention (Frey 2019). Most trees form a symbiosis with one of two types of mycorrhizal fungi: arbuscular mycorrhizae (AM) or ectomycorrhizae (EcM). There is accumulating evidence that SOM differs between ecosystems dominated by these mycorrhizal types (Averill et al. 2014, Zhu et al. 2018). Specifically, bulk soil C:Nitrogen (N) ratios are generally greater in EcM relative to AM ecosystems (Averill et al. 2014, Zhu et al. 2018, Cotrufo et al. 2019) with EcM ecosystems having higher soil C concentrations (Averill et al. 2014, Wu et al. 2022) but storing less N (Zhu et al. 2018). While AM forests can store less topsoil C than EcM forests, they can store more deep soil C leading to greater soil C storage overall (Craig et al. 2018).

Not all soil C has the same long-term sequestration potential. Soil C exists on a continuum of transit times (Sierra et al. 2017) based on its molecular composition, degree of decomposition, and accessibility to microbes (Schmidt et al. 2011, Lehmann et al. 2020). Conceptually, soil organic matter (SOM) is often split into multiple pools, which differ in their stabilization

mechanisms and susceptibility to microbial decay. In a broad sense, there is particulate organic matter (POM), which can be unprotected or occluded within aggregates, and mineral-associated organic matter (MAOM), in which organic matter is sorbed onto mineral surfaces or complexed with metals (Lavallee et al. 2020). MAOM fractions have longer radiocarbon-based ages than POM fractions indicating resistance to microbial decomposition (Heckman et al. 2022) and are generally less responsive to global changes like soil warming (Soong et al. 2021, Rocci et al. 2021). The proportion of POM and MAOM in SOM differs with the dominant mycorrhizal association of an ecosystem (Craig et al. 2019, Cotrufo et al. 2019, Wu et al. 2022). EcM-dominated forests tend to have a greater proportion of their SOM stored in POM pools relative to AM-dominated forests. These differences in the proportion of protected SOM could affect the susceptibility of soil C stocks to global change-driven losses.

Two related explanations exist for why SOM differs between forests dominated by AM- or EcM-associated tree species. One hypothesis, which is not directly related to the fungi themselves, rests on differences in litter chemistry between host plant species (Lin et al. 2017). AM leaf litter tends to decompose faster than EcM litter (Cornelissen et al. 2001, Midgley et al. 2015, Sun et al. 2018, Keller and Phillips 2019). High quality, easier-to-decompose litter is utilized more efficiently by microbes, which leads to more OM in microbial necromass that ultimately sorbs to soil minerals (Cotrufo et al. 2013, Córdova et al. 2018). If litter chemistry is the dominant control of MAOM formation, we would expect larger MAOM pools in AM systems and larger POM pools in EcM systems. This pattern has been observed across European grasslands, agricultural lands, and forests (Cotrufo et al. 2019) and forests in the southeastern United States (Craig et al. 2019). A second hypothesis is based on how the fungal symbionts acquire nutrients. While both AM and EcM fungi can acquire mineral forms of N, some EcM fungi also act as

"decomposers" by producing degradative enzymes (e.g. peroxidases) to speed N release from OM (Lindahl and Tunlid 2015). This organic N mining by EcM fungi can limit the activity of saprotrophs via competition for N through the hypothesized 'Gadgil effect' (Gadgil and Gadgil 1975, Averill and Hawkes 2016). A less active saprotrophic community could also lead to more SOM remaining in the partially decomposed POM pool in EcM-dominated systems.

In spite of these developing theories, we lack systematic tests of the mechanisms by which mycorrhizal associations affect SOM. Mycorrhizal associations are being incorporated into models of coupled C and N cycles (e.g., Sulman et al. 2017), offering an important step towards projecting future forest C storage as global change alters species composition (Jo et al. 2019). Such a consideration is critical in the eastern United States, where AM-associated tree species are increasing in dominance due to climate change, N deposition, and fire exclusion (Averill et al. 2018, Jo et al. 2019). However, accurately incorporating mycorrhizal fungi into biogeochemical models depends on identifying the most important mechanisms responsible for differences in SOM quality and quantity. For example, if litter quality is the driver of AM-EcM differences in SOM, models that consider plant functional types or species-specific effects (e.g., Crowley and Lovett 2017) might be sufficient for capturing these mycorrhizal differences. If, on the other hand, EcM fungal functional traits are the main driver, we may need to account for functional differences within the EcM fungal guild. Not all EcM fungi have the same capacity to oxidize organic matter and liberate N (Bödeker et al. 2009). In this case, the inclusion of the AM/EcM dichotomy into models such as CORPSE (Sulman et al. 2019), may be insufficient unless they include more granularity in the diversity of fungal traits among EcM fungi.

Most prior studies of mycorrhizal effects on SOM have focused on differences between AM and EcM dominated forests (e.g., Cotrufo et al. 2019, Keller et al. 2021) or across gradients

of EcM dominance (e.g., Craig et al. 2019). While revealing the aforementioned SOM differences between AM and EcM systems, many of these studies have not investigated species effects beyond the AM/EcM dichotomy. Here, we investigated how mycorrhizal associations are related to soil C and N across four sites in the eastern U.S. broadleaf forest biome, which capture broad variability in climate and tree species. Our sites represented each of the four ecoregions in the eastern United States temperate forest—warm continental (New Hampshire), hot continental (Wisconsin), Prairie (Illinios), and subtropical (Georgia)—described in Jo et al. (2019). This breadth naturally provided tree species diversity and allowed us to also investigate how soil C and N are related to canopy tree and EcM fungal community composition. Specifically, we analyzed the effects of mycorrhizal association, canopy tree family, and EcM fungal taxa on the proportion of C and N associated with soil minerals (MAOM) and bulk soil C:N. Furthermore, we explored potential mechanisms by which mycorrhizal associations and species affect soil C and N by examining relationships with leaf litter quality and the enzymatic potential of EcM fungi. We hypothesized that AM systems would have a larger proportion of soil C and N in mineral-associations and a lower bulk soil C:N than EcM systems, but that the magnitude of the differences would depend on the EcM taxa present.

Methods

Study Sites

We collected soils across gradients of EcM basal area in four forested sites across the eastern United States (Table 1): Flambeau River State Forest, Wisconsin (45.62°N, 90.79°W, 122,000 m^2 area; WI), Allerton Park and Recreation Center in Monticello, Illinois (39.99°N, 88.66°W, 103,000 m^2 area; IL), Chattahoochee National Forest (34.99°N, 83.56°W, 245,000 m^2 area and 34.96°N, 83.48°W, 15,000 m^2 area; hereafter referred to as GA), and Norwich,

Vermont/Hanover, New Hampshire (43.72°N, 72.35°W, 20,000 m^2 area and 43.72°N, 72.16°W, 60,000 m^2 area; hereafter referred to as NH). These sites can all be classified as hardwood forests, except for the NH location, which is a mixed hardwood forest. Historical rates of N deposition have been similar across sites with recent decreases in NH relative to the other sites (Table 1). At each site, we collected soils from eight to twelve 10-m radius plots across a gradient of EcM basal area. Plots were spaced at least 25 m apart. Within each plot, we measured the diameter at breast height (1.3 m) of all trees greater than 2 cm in diameter and identified each tree to species where possible, and genus where not. We assigned all trees as EcM or AM based on their genus (Appendix S1: Table S1). The basal area of *Populus*, known to associate with both EcM and AM fungi, was assigned as 50% EcM. In GA, <6% of the total basal area was occupied by the ericaceous tree, *Oxydendron arboreum*. Given that ericoid mycorrhizae are more functionally similar to EcM than to AM, we assigned the basal area of this genus to EcM, but we also present data without including them as EcM (Appendix S1). The understory did not include ericaceous shrubs at any sites except GA, where thickets of evergreen ericaceous shrubs were avoided when setting up plots.

Soil Collection and Characterization

At each site, we collected soil cores of the top 10 cm of mineral soil after removing the O horizon. We combined mineral soil cores collected from two spots at the center of our circular plots using a 5 cm diameter stainless steel corer in NH. At the other sites (WI, IL, and GA), the 10-m radius plots were divided into four sections using cardinal directions. Within each section, five cores were randomly taken using a 1 cm diameter soil probe. All cores from each plot were then combined and homogenized for analysis. Soil samples were sieved through a 2 mm mesh. A subsample was dried at 60°C and ground to measure %C and %N on an Elemental Analyzer

(Costech ECS 4010 for NH soils and a Carlo Erba NA1500 for other soils). We took subsamples for density fractionation (dried at 60°C), and subsamples for DNA extraction (frozen at -80°C).

The dried soil was separated by density into fine POM, occluded POM, and MAOM pools (Sollins et al. 2006, 2009). We added 75 ml of 1.7 mg cm^{-3} of low C/N sodium polytungstate (SPT) solution to 20 g of dried soil. We tested the density of 1.7 mg cm^{-3} across all our sites to ensure it resulted in good separation of POM and MAOM and left few soil minerals in the POM fraction. The SPT/soil mixture was then shaken for five minutes on a reciprocating shaker before being centrifuged in a swinging bucket rotor for one hour (4710 *g* and 20°C). The duration of centrifugation was determined based on the chosen SPT density so that no dense particles with diameters >0.8 μm remained in solution. The free light fraction (fine POM) was then aspirated from the centrifuge tube, filtered through a 0.8 μm polycarbonate filter, and rinsed with deionized water until the filtrate was the density of water. The fine POM was then rinsed off the filter into a drying tin. The remaining soil and SPT solution were shaken on a reciprocating shaker for five minutes, sonicated for two minutes (while in an ice bath to prevent warming), and centrifuged again for an hour before aspirating the occluded light fraction (occluded POM), filtering, and rinsing it as above. Lastly, the dense fraction (MAOM) was rinsed and centrifuged until the supernatant was the density of water before transfer to a drying tin. All fractions were dried, weighed, ground, and analyzed by Elemental Analyzer (Costech ECS 4010) for %C and %N.

Fungal ITS Sequencing and Community Analysis

Soil DNA extractions were performed on 1 g of fresh soil using the Omega Bio-tek E.Z.N.A. Soil DNA kit following the manufacturer's protocol. Fungal communities were characterized by sequencing the ITS2 region of the fungal rRNA gene using forward ITS3-KYO2 (Toju et al. 2012) and reverse ITS4 (White et al. 1990) primers with added Nextera read primer

sequences for the first PCR reaction (Lankau and Keymer 2016). Duplicate PCR reactions were conducted under the conditions outlined in Caporaso et al. (2011). The second round of PCR added the P5 and P7 flow cell adapters to prepare the library for sequencing on an Illumina MiSeq, along with an external set of sample barcodes located between the flow cell adaptors and read primers. Amplicons were cleaned with the Omega BioTek E-Z 96 Cycle Pure kit. Purified products were quantified using a Qubit 2.0 fluorometer with the Qubit dsDNA HS assay (Thermo Scientific, Grand Island, NY). Amplicons were pooled at equal concentration and sequenced on an Illumina MiSeq using V3 chemistry using paired-end sequencing (300 cycles).

Barcode sequences were separated by the University of Wisconsin-Biotechnology center, filtered for quality, and assigned to amplicon sequence variants (ASV's, equivalent to 100% identity operational taxonomic units) using the DADA2 program (Callahan et al. 2016) as implemented in the QIIME2 pipeline. Three samples from NH and four from GA produced too few quality sequence reads to be included in our analyses. Non-singleton ASVs were identified to the lowest confident taxonomic level using the naïve Bayesian classifier RDP and the UNITE database for ITS reads (Nilsson et al. 2019). We used the FUNGUILD database to assign ASVs to functional groups (Nguyen et al. 2016). Based on the annotations provided by FUNGUILD, we assigned all ASVs to one of five broad putative categories: AM fungi, EcM fungi, plant pathogens, saprotrophs, and other. If an ASV was assigned to multiple categories that included EcM fungi, it was assigned to EcM fungi. All subsequent statistical analyses were performed on the EcM fungi subset. We assigned peroxidase capabilities to EcM fungal genera using the approach in Argiroff et al. (2022). We recognize that assigning all species in a genera as "peroxidase-producing" based on one species belonging to that genus containing peroxidase gene copies is a large assumption;

however, it is currently our best estimate awaiting further research into fungal genomes and function.

Tree community-weighted litter properties

We searched the literature for the leaf litter decomposition constants (*k*) and C:N ratios of the tree species found at our sites. We conducted these searches in *Web of Science* and *Google Scholar* using the keywords such as "litter," "decomposition," "decay," and "C:N," as well as the latin and common names for each tree species. We also extracted decomposition constants and C:N ratios from the Long-Term Inter-site Decomposition Experiment Team (LIDET; Harmon et al. 2009) and the TRY Plant Trait Database (Kattge et al. 2020). We extracted data from graphs using WebPlotDigitizer when actual numbers were not available in the text or tables. For studies that reported mass loss over time, but not the k value, we used R to estimate the decomposition constant by fitting a single exponential decay function to the data. C:N mass ratios were calculated manually if they were not directly reported. We averaged data for litter chemistry and decomposition per species per published study, except for LIDET data, which was not averaged due to the continental study area. We noted discrepancies in variables such as mesh size, location of study, ecosystem, and state of leaf senescence. The data were then averaged by species. For species with insufficient data by species, we assigned an average based on tree family. We then calculated an average litter C:N and k value for each plot by weighting the values by the basal area of each species.

Statistical Analyses

Tree communities were compared among the sites using a Principal Components Analysis (PCA). There were 16 plant families represented in our sites, but we chose to run the PCA using the nine most dominant plant families based on their total basal area (>0.8 m^2) to avoid biasing the

results due to zero inflation. A PCA was chosen so that we could use the principal components as predictors of SOM properties. We ran the PCA in R (R Core Team 2021) using the prcomp function and graphed the results with ggbiplot. EcM fungi communities were compared among sites using bray-curtis dissimilarity indices in several ways including a a permutational multivariate analysis of variance (MANOVA), and an analysis of similarity (ANOSIM) in R (vegan package; Oksanen et al. 2020). Site dispersions were compared using the betadisper function. Site EcM fungi communities were compared using nonmetric multidimensional scaling (NMDS). Finally, relative abundances were calculated to examine the dominant EcM fungal taxa within sites.

We first used multiple linear regressions to examine the relationship between our SOM properties, bulk C:N and the proportion of C and N in MAOM, and EcM basal area and its interaction with site. We then used multiple regressions to examine the relationship between the SOM properties with the first three principal components of the plant family PCA and site. For the proportion of C in MAOM and bulk C:N, the relationships with the predictors were not linear, so a general additive model (GAM) was performed using the gamm function in the mgcv package in R (Wood 2011). For the models with the PCA predictors, we compared all combinations of model predictors and selected the most parsimonious set of predictors based on the model with the lowest corrected AIC (AICc). Additionally, we ran linear models to test how the absolute abundance of MAOM C and POM (fine plus occluded) C changed with the PCA predictors and site.

We used multiple regressions to test how SOM properties related to the basal area-weighted litter C:N and to the decomposition constants including an interaction with site. All regressions were linear, except for the relationship between soil and litter C:N where a GAM was used. For all regressions, residuals were checked for normality and homogeneity of variance. A constant

variance structure based on site was used in all models. We also explored the relationships between potential plant-related predictors of SOM properties (EcM basal area, the second principal component of the dominant plant family PCA, basal-area weighted litter k and C:N) using correlations.

We tested the correlation of EcM fungal community composition with each SOM property, with the tree community, and with EcM basal area using Mantel tests from the vegan package in R (permutations=9999). The EcM fungal community and plant community dissimilarity matrices were based on Bray-Curtis while for each SOM property and EcM basal area a dissimilarity matrix based on euclidean distances was used. Lastly, we regressed the proportion of C and N in MAOM against the relative abundance of peroxidase-capable EcM. All of the data used here can be accessed via the ESS-DIVE repository (Hicks Pries et al. 2022), and the code for performing the statistical analyses and producing the graphs is available on Figshare (Hicks Pries 2022).

Results

Tree and EcM Fungi Communities

The sites differed in both their dominant tree communities and their EcM fungal communities. Forty tree species were present among all sites, with 10, 13, 13, and 19 species found at sites in WI, NH, IL, and GA, respectively (Appendix S1: Table S1). A PCA using the basal area of the 9 dominant plant families showed a strong separation among the sites (Fig. 1A). The first principal component explained 28.8% of the variation and separated the more northern sites (in NH and WI) from the more southern sites (in GA and IL). This component was driven by the basal area of Betulaceae (birches) relative to the basal area of Juglandaceae (EcM hickories and AM walnuts), Ulmaceae (elms), and Fagaceae (oaks and beeches; Appendix S1: Table S2). The second principal component explained 16.6% of the variation and separated sites in NH and GA from the

sites in WI and IL. This component was driven by the basal area of Pinaceae (pines and hemlocks) and Fagaceae, which were more prevalent at the NH and GA sites.

The composition of EcM fungal communities also differed among sites as demonstrated by NMDS (Fig. 1B). Both graphs showed that sites in NH and GA had more similar communities than the sites in IL and WI. According to an ANOSIM of the EcM fungal communities, between group differences were greater than within group differences ($R=0.78$, $p<0.0001$, permutations=9999). A permutational MANOVA also showed significant differences in EcM fungal communities between sites ($p<0.0001$, df=3, permutations=9999). The communities were more dispersed in NH than at other sites (test of group homogeneity; $p=0.0001$), which may have affected the results; however, the NMDS plot showed that the site centroids still differed. Dominant EcM taxa (based on relative abundance) differed across sites; *Inocybe* and *Tuber* were more abundant in WI and IL while *Cenococcum*, *Piloderma* and *Tricholoma* were more abundant in GA and NH (Appendix S1: Fig. S1).

Soil Organic Matter

Soil OM properties were significantly related to the percentage of EcM basal area only at the GA and NH sites; there was no significant relationship between SOM properties at the WI and IL sites (Fig. 2). The proportion of C in MAOM decreased as the percentage of EcM basal area increased in NH and GA (Site x EcM interaction, $f=5.084$, df=3, $p=0.0058$), as did the proportion of N in MAOM (Site x EcM interaction, $f=3.22$, df=3, $p=0.0364$). Similarly, while there was no significant relationship between soil C:N and the percentage of EcM basal area at the WI and IL sites, soil C:N significantly increased as the percentage of EcM basal increased at the NH and GA sites (Site x EcM interaction, $f=7.236$, df=3, $p=0.0009$). Assigning *Oxydendron* basal area as non-EcM did not affect the results (Appendix S1: Fig. S2)

Relating SOM properties to the tree communities, the best fit model for all properties included the second principal component from the PCA of the dominant plant families (PC2; i.e., the basal area of trees in the Pinaceae and Fagaceae) and site as significant predictors (Fig. 2). The proportion of C and N in MAOM decreased significantly as the basal area of Pinaceae and Fagaceae increased (estimated df=3.69, f=4.535, p=0.006 for C and df=1, f=13.409, p=0.0009 for N)). Lastly, soil C:N increased significantly with the basal area of Pinaceae and Fagaceae, and like with C in MAOM, the increase only occurred when values of PC2 were greater than 0 (estimated df=4.06, f=20.12, p<0.0001). There was a significant site effect for all SOM properties, but no interaction of site with the basal area of Pinaceae and Fagaceae. Plotting the residuals of the GAM models by site visually demonstrated that fitting separate smoothing curves by site was not needed, and the best fit of the linear soil C:N model based on AICc did not include the interaction. Decreases in the proportion of MAOM as the basal area of Pinaceae and Fagaceae increased were driven by increases in the abundance of POM-C in NH (df=3, f=2.8, p=0.055) and decreases in the abundance of MAOM-C in GA (df=3, f=2.1, p=0.12); the abundance of POM-C and MAOM-C remained relatively constant in IL and WI (Appendix S1: Fig. S3).

To explore mechanisms of how tree identity could affect SOM properties, we related SOM properties to characteristics of the aboveground litter at each site using basal area-weighted litter C:N and decomposition constants (Appendix S1: Fig. S4). There were no significant relationships between the basal area-weighted decomposition constant (*k*) and the SOM properties (litter *k* effect, df=1, p's>0.11), nor were there any significant interactions (litter *k* x site effect, df=3, p's>0.18). There were significant relationships between the basal area-weighted litter C:N and some SOM properties. The proportion of C and N in MAOM decreased as the basal area-weighted litter C:N increased (litter C:N effect, df=1, p's<0.0005) without significant interactions between

site and litter C:N (litter C:N x site effect, df=3, p's>0.55). Soil C:N was not significantly related to the basal area-weighted litter C:N (litter C:N effect in GAM, estimated df=1, p=0.87) but was significantly related to site with GA and NH having higher soil C:N than IL and WI (site effect, df=3, p<0.0001). Overall, the basal area of Pinaceae and Fagaceae (PC2) was more highly correlated with the community-averaged litter properties than the percentage of EcM basal area (Appendix S1: Fig. S5). Litter C:N increased (df=36, r=0.47, p=0.003) and litter *k* decreased (df=36, r=-0.41, p=0.009) as the basal area of Pinaceae and Fagaceae increased. There were no significant correlations of community litter properties with EcM basal area.

Investigating the correlation of EcM fungal communities with SOM, the dissimilarity of the three SOM properties were significantly correlated with the compositional variation (beta diversity) of the EcM fungi (Appendix S1: Fig. S6). Specifically, the proportion of C and N in MAOM across our sites decreased significantly with increasing relative abundances of peroxidase-capable EcM (Fig. 3; df=1, p's<0.002). Soil C:N was not related to the abundance of peroxidase-capable EcM (p=0.18). Similar to litter properties, the compositional variation of the EcM fungal communities was not significantly correlated with differences in the EcM basal area (mantel r=-0.004, p=0.475), but was significantly correlated with the compositional variation of the tree communities (mantel r=0.3428, p=0.001; Appendix S1: Fig. S7).

Discussion

Only half of our sites demonstrated the expected relationship of declining MAOM fractions and increasing soil C:N ratios as EcM basal area increased, and this result was not due to soil minerals (Appendix S1: Figure S8). At first glance, the fact that our findings did not conform to mycorrhizal relationships observed at regional (Craig et al. 2019), continental (Jo et al. 2019, Cotrufo et al. 2019), and global scales (Wu et al. 2022) may seem surprising. However, a closer

look at the meta-analyses of Cotrufo et al. (2019) and Wu et al. (2022) reveals large variability around the means and medians of soil C:N and the mineral-associated fraction, particularly for EcM-dominated ecosystems. In these studies, the range of soil values in EcM ecosystems exceed both the upper and lower limits of those in AM ecosystems, suggesting that an understanding of diverse EcM effects on soils could be more informative than the AM/EcM dichotomy. We used our data to explore the potential drivers of this large variability within EcM ecosystems. Understanding what drives this variability may help elucidate the mechanisms by which mycorrhizal types affect ecosystem processes like SOM storage (Averill et al. 2014, Zhu et al. 2018, Craig et al. 2018), N cycling (Phillips et al. 2013, Lin et al. 2022), and responses to global change (Terrer et al. 2021). Mechanisms that have been invoked to explain the differences between AM and EcM ecosystems include differences in leaf litter quality (Sun et al. 2018, Keller and Phillips 2019), rhizodeposition (Keller et al. 2021), fungal necromass decomposition (Langley and Hungate 2003), and nutrient acquisition strategies (Averill et al. 2014; Fig. 4). Here, we focus on how the functional variability of different EcM-associating tree species and EcM fungi may explain the observed pattern across our sites and thus help explain the range in soil characteristics among EcM ecosystems.

The role of tree characteristics

A diversity of plant families were represented across our sites, allowing us to explore how species variability affects the EcM/AM dichotomy. In terms of EcM-associated families, Pinaceae and Betulaceae dominated the NH plots, Fagaceae and Pinaceae dominated the GA plots, Fagaceae and Juglandaceae (*Carya* genus) dominated the IL plots, and Betulaceae and Malvaceae dominated the WI plots. This variation in EcM trees across our sites may have contributed to the lack of consistent relationships between EcM basal area and soil properties because leaf litter properties

did not follow expectations. We expected litter quality would decrease as EcM basal area increased because generally EcM leaf litter has a slower decay rate than AM leaf litter (Sun et al. 2018, Keller and Phillips 2019). However, in our sites EcM basal area was not correlated with either basal-area weighted litter C:N or decomposition constants. It should be noted that these metrics were only an approximation of overall litter quality; they assume that litterfall scales with basal area and that average literature values are representative of the litter quality at our sites, which can shift depending on climate and nutrient availability.

Litter quality may have contributed to differences in soil properties among our sites but not through the lens of the AM/EcM dichotomy. Instead, this relationship was driven by the basal area of trees in the Pinaceae and Fagaceae, which were significantly related to soil properties through the second principal component of the plant family PCA. In contrast to EcM basal area, PC2 was significantly correlated with the weighted litter C:N and decomposition constants. Trees in the Pinaceae and Fagaceae families had the lowest-quality leaf litters out of the plant families in this study, with the smallest average decay constants (0.36±0.05, n=17 and 0.51±0.05, n=31, respectively) and high average C:N values (76±8, n=9 and 64±8, n=9, respectively). The sites that had the most Pinaceae and Fagaceae (NH and GA) had significantly greater soil C:N than the WI and IL sites. Soil C:N values generally decrease from the values of plant litter (ranged from 19 to 91 in the data compiled for this study) to the values of microbial biomass (≈8-9) as microbes decompose SOM because more N remains in the soil relative to C, which is lost via respiration (Cleveland and Liptzin 2007). Higher soil C:N in Pinaceae- and Fagaceae- dominated plots suggests that their low-quality leaf litter slowed decomposition. Supporting this point, the proportion of C and N in MAOM decreased as plot-averaged litter C:N increased. Labile litter is more likely to become MAOM because it can be more efficiently incorporated into microbes

during decomposition, which can then become sorbed to soil minerals when the microbes die (Cotrufo et al. 2013). Overall, our results demonstrate that plant identity affects soil properties but not necessarily based on their mycorrhizal associations. This result calls into question the utility of AM and EcM designations in models and meta-analyses. In order to assign AM or EcM types, the plant species must be known, which makes it just as feasible to consider how plant phylogeny helps explain patterns in SOM. Important information is lost when the diversity of plants is reduced to two categories. Our study suggests plant family might be an appropriate level of generalization.

Belowground inputs are another potential reason plant families were a better predictor of soil properties than EcM basal area. Root inputs are often larger contributors to SOM than aboveground plant inputs (Rasse et al. 2005, Jackson et al. 2017, Sokol et al. 2019). However, in forests, retention of leaf and root litter inputs can be similar within the top of the mineral soil (Hicks Pries et al. 2017, Keller et al. 2021). Leaf and root litter quality do not necessarily follow the same patterns within mycorrhizal types (Taylor et al. 2016, Sun et al. 2018) but do generally covary within a species (Freschet et al. 2012), which might make plant identity a better predictor of soil properties than mycorrhizal type. Notably, one meta-analysis that found mycorrhizal type was a significant predictor of root decay with EcM roots decaying more slowly than AM roots had a preponderance of data points from the EcM orders Fagales and Pinales (See et al. 2019). The low-quality root litter of Pinaceae and Fagaceae could have contributed to the relationship between soil properties and the basal area of Pinaceae and Fagaceae (PC2) much like the low-quality leaf litter discussed above.

Rhizodeposits are another belowground source of SOM that can differ among tree species. Higher rates of root exudation should fuel microbial activity in the rhizosphere, promoting the formation of MAOM (Sokol et al. 2019). Root-derived C accumulation was higher among AM vs.

EcM tree seedlings in a mesocosm study (Wurzburger and Brookshire 2017), and greater over two growing seasons in AM-dominated forests than EcM-dominated forests (Keller et al. 2021). This accumulation of root-derived C also resulted in greater root-derived mineral-associated C in AM-dominated forests, which matches the pattern of increased MAOM as AM basal area increased at our NH and GA sites. In Keller et al. (2021), the largest differences were found between AM and Pinaceae- versus Fagaceae-dominated sites. Collectively, our results and these studies illuminate that plant family may be a better predictor of MAOM and soil C:N than mycorrhizal association regardless of the organic matter source.

The role of fungal characteristics

The forests represented in our sites differed not only in the phylogeny of dominant EcM-associated plants, but also in the species of the EcM fungi themselves. EcM fungal communities were more similar in the NH and GA sites, where we observed the expected pattern in SOM properties with EcM basal area, and were different from the IL and WI sites where the patterns were not observed. Dominant EcM taxa differed with *Inocybe* and *Tuber* having higher abundance in WI and IL and *Cenococcum*, *Piloderma* and *Tricholoma* having higher relative abundance in GA and NH. These differences in dominant species may also affect SOM given that EcM fungi have more functional variability than AM fungi, having independently evolved over 80 times (Pellitier and Zak 2018). We found evidence for this connection between species and function across our sites, where the compositional variation of the EcM fungal communities was significantly correlated with the proportion of C and N in mineral-associations and soil C:N. EcM fungal species differ in their growth form (Agerer 2001), their affinity for N-rich soils (Cox et al. 2010, Lilleskov et al. 2011), the degree to which their hyphae are melanized (Silleti et al. 2017),

and their capacity to produce extracellular enzymes (Bödeker et al. 2009, Lindahl and Tunlid 2015). These traits can affect SOM cycling both directly and indirectly.

EcM fungi can be categorized by 'exploration type' based on the distance traveled from the root tip by their hyphae, the density of their rhizomorphs, and hydrophobicity of their hyphae (Agerer 2001). The categories include contact with few emanating hyphae, medium distance, and long-distance exploration types (Agerer 2001). These categories may represent different foraging strategies and have been shown to correlate with soil nutrients and soil C stocks (Clemmensen et al. 2021). For example, species with medium distance fringe and mat exploration types, hypothesized to access organic N sources, decline in response to N deposition (Lilleskov et al. 2011). These different growth forms may have ramifications for the volume of soil influenced by hyphae and the decay rates of their necromass (Fernandez et al. 2016). EcM fungi can have 175,000 cm of hyphae per cm^3 soil, which is several orders of magnitude greater than the estimated 2400 cm of AM hyphae per cm^3 soil (See et al. 2022). Therefore, mycorrhizal hyphae facilitate the formation of MAOM by delivering plant-derived C to mineral surfaces via exudates and necromass (See et al. 2022). In this study, GA and NH sites had greater relative abundances of medium-distance fringe exploration types, *Piloderma* and *Tricholoma*, while IL and WI had greater relative abundances of contact/short distance, non-rhizomorph-forming exploration types *Inocybe* and *Tuber*. While it is unknown how these exploration types affect SOM cycling, longer distance, rhizomorph-forming EcM fungi may have led to more EcM fungal influence in GA and NH sites where the expected SOM patterns with increasing EcM basal area were seen.

Mycorrhizal fungi are an important source of SOM via their necromass (Clemmensen et al. 2013, Liang et al. 2019). Necromass inputs are a function of hyphal biomass and turnover rates. EcM fungal hyphae turnover more slowly than AM hyphae, but species-specific data for EcM

fungi are lacking (See et al. 2022). Fungal necromass decomposition depends on the degree to which the fungal tissues are melanized with melanized necromass decomposing more slowly (Fernandez and Koide 2014). Overall, EcM hyphae are more melanized than AM hyphae, but EcM species differ in their degree of melanization (Siletti et al. 2017). For example, among species colonizing the Pinaceae, *Geospora* sp. are highly melanized while *Suillus* sp. are not (Fernandez et al. 2016). Soil melanin contents are positively related to soil C (Siletti et al. 2017). Since melanin retards decomposition, the dominance of EcM species with high melanin would likely decrease the proportion of MAOM and increase soil C:N. While not dominant, the highly melanized *Cenococcum* (Siletti et al. 2017) did have greater relative abundances in the NH and GA sites relative to the WI and IL sites.

Lastly, EcM species differ in their ability to produce extracellular enzymes, particularly peroxidases (Bödeker et al. 2009). *Cortinarius*, *Russula*, and *Lactarius* are the main genera that have peroxidase genes, and *Cortinarius* are correlated with high peroxidase activity in soils (Bödeker et al. 2014). Across boreal and northern temperate ecosystems, C storage in organic horizons is negatively correlated with the abundance of peroxidase-producing EcM fungi (Clemmensen et al. 2013, Lindahl et al. 2021, Argiroff et al. 2022), perhaps due to their direct contribution to organic matter decomposition (Argiroff et al. 2022). In our study, the proportion of MAOM across our sites decreased significantly with increasing relative abundances of peroxidase-capable EcM (*Piloderma*, *Cortinarius*, *Russula*, and *Lactarius*). This pattern suggests that EcM fungi with peroxidases may suppress decomposition, leading to a higher proportion of POM. One mechanism for this suppression is that peroxidase-capable EcM fungi may induce saprotrophic N limitation by mining N from organic matter (Averill and Hawkes 2016). While experiments testing whether EcM fungi suppress decomposition (i.e., the Gadgil effect) have had

equivocal results (e.g., Brzostek et al. 2015, Lang et al. 2021), one recent study suggested the Gadgil effect predominantly occurs in systems with high lignin:N litter inputs (Smith and Wan 2019), which would apply to the GA and NH sites where Pinaceae and Fagaceae dominated the EcM plots and where peroxidase-capable EcM fungi were more prevalent. Differences in EcM fungi's ability to mine N from SOM is a potential, yet unexplored, reason for the context-dependency of the Gadgil effect.

Conclusion

This observational study shows that differences in SOM driven by the AM-EcM dichotomy are dependent on tree and fungal species. Thus, functional trait diversity within plant host and fungal associates modulates soil C and N cycling. The expected patterns of increasing soil C:N and decreasing MAOM with increasing EcM basal area were only observed within sites where: 1) the dominant EcM-associated trees were in the Pinaceae or Fagaceae and 2) the dominant EcM fungi had medium distance exploration types, more melanized tissues, and a greater potential to produce peroxidases. The extent to which the identity of the plants versus the identity of the mycorrhizal fungi are driving these patterns separately or co-varying as paired trait syndromes is not known. Neither is the extent to which the patterns observed here in the top 10 cm of mineral soil are reflected across the rest of the soil profile. Careful experiments are needed to disentangle the mechanisms driving these patterns and to test the relative influence of plant effects (leaf and root litter quality, root exudation) and fungal effects (growth form, necromass quality, and extracellular enzyme capacity) on SOM. These experiments will also help illuminate the mechanisms causing patterns across AM- and EcM-dominated systems more generally. How these species interactions affect SOM cycling, particularly the partitioning of SOM to mineral-

associated pools that are less vulnerable to climate change, is an important question as global change causes shifts in the species composition of forests.

Acknowledgements

We would like to thank Cassandra Allsup for field assistance and Fernando Montaño Lopez for assistance with obtaining N deposition estimates. This work was supported as part of the Terrestrial Ecosystem Science Program by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under contract DE-SC0020228.

Conflict of Interest

The authors declare they have no conflict of interest.

References

Agerer, R. 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114.

Argiroff, W. A., D. R. Zak, P. T. Pellitier, R. A. Upchurch, and J. P. Belke. 2022. Decay by ectomycorrhizal fungi couples soil organic matter to nitrogen availability. Ecology Letters 25:391–404.

Averill, C., M. C. Dietze, and J. M. Bhatnagar. 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. Global Change Biology 24:4544–4553.

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

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

Bödeker, I. T. M., K. E. Clemmensen, W. de Boer, F. Martin, Å. Olson, and B. D. Lindahl. 2014. Ectomycorrhizal Cortinarius species participate in enzymatic oxidation of humus in

northern forest ecosystems. New Phytologist 203:245–256.

Bödeker, I. T. M., C. M. R. Nygren, A. F. S. Taylor, Å. Olson, and B. D. Lindahl. 2009. ClassII peroxidase-encoding genes are present in a phylogenetically wide range of ectomycorrhizal fungi. The ISME Journal 3:1387–1395.

Brzostek, E. R., D. Dragoni, Z. A. Brown, and R. P. Phillips. 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. New Phytologist 206:1274–1282.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature methods 13:581.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522.

Clemmensen, K. E., A. Bahr, O. Ovaskainen, A. Dahlberg, A. Ekblad, H. Wallander, J. Stenlid, R. D. Finlay, D. A. Wardle, and B. D. Lindahl. 2013. Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. Science 339:1615–1618.

Clemmensen, K. E., M. B. Durling, A. Michelsen, S. Hallin, R. D. Finlay, and B. D. Lindahl. 2021. A tipping point in carbon storage when forest expands into tundra is related to mycorrhizal recycling of nitrogen. Ecology Letters 24:1193–1204.

Cleveland, C. C., and D. Liptzin. 2007. C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252.

Córdova, S. C., D. C. Olk, R. N. Dietzel, K. E. Mueller, S. V. Archontouilis, and M. J.

Castellano. 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology and Biochemistry 125:115–124.

Cornelissen, J., R. Aerts, B. Cerabolini, M. Werger, and M. van der Heijden. 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia 129:611–619.

Cotrufo, M. F., M. G. Ranalli, M. L. Haddix, J. Six, and E. Lugato. 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12:989–994.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial Efficiency-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.

Cox, F., N. Barsoum, E. A. Lilleskov, and M. I. Bidartondo. 2010. Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecology letters 13:1103–1113.

Craig, M. E., N. Lovko, S. L. Flory, J. P. Wright, and R. P. Phillips. 2019. Impacts of an invasive grass on soil organic matter pools vary across a tree-mycorrhizal gradient. Biogeochemistry 144:149–164.

Craig, M. E., B. L. Turner, C. Liang, K. Clay, D. J. Johnson, and R. P. Phillips. 2018. Tree mycorrhizal type predicts within-site variability in the storage and distribution of soil organic matter. Global Change Biology.

Crowley, K. F., and G. M. Lovett. 2017. Effects of nitrogen deposition on nitrate leaching from

forests of the northeastern United States will change with tree species composition. Canadian Journal of Forest Research 47:997–1009.

Fernandez, C. W., and R. T. Koide. 2014. Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. Soil Biology and Biochemistry 77:150–157.

Fernandez, C. W., J. A. Langley, S. Chapman, M. L. McCormack, and R. T. Koide. 2016. The decomposition of ectomycorrhizal fungal necromass. Soil Biology and Biochemistry 93:38–49.

Freschet, G. T., R. Aerts, and J. H. C. Cornelissen. 2012. A plant economics spectrum of litter decomposability. Functional Ecology 26:56–65.

Frey, S. D. 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annual Review of Ecology, Evolution, and Systematics 50:237–259.

Gadgil, P. D., and R. L. Gadgil. 1975. Suppression of litter decomposition by mycorrhizal roots of Pinus radiata. New Zealand Forest Service.

Griscom, B. W., J. Adams, P. W. Ellis, R. A. Houghton, G. Lomax, D. A. Miteva, W. H. Schlesinger, D. Shoch, J. V. Siikamäki, and P. Smith. 2017. Natural climate solutions. Proceedings of the National Academy of Sciences 114:11645–11650.

Harmon, M. E., W. L. Silver, B. Fasth, H. U. A. Chen, I. C. Burke, W. J. Parton, S. C. Hart, W. S. Currie, and Lidet. 2009. Long-term patterns of mass loss during the decomposition of leaf and fine root litter: an intersite comparison. Global Change Biology 15:1320–1338.

Heckman, K., C. E. Hicks Pries, C. R. Lawrence, C. Rasmussen, S. E. Crow, A. M. Hoyt, S. F. von Fromm, Z. Shi, S. Stoner, C. McGrath, J. Beem-Miller, A. A. Berhe, J. C. Blankinship, M. Keiluweit, E. Marín-Spiotta, J. G. Monroe, A. F. Plante, J. Schimel, C.

A. Sierra, A. Thompson, and R. Wagai. 2022. Beyond bulk: Density fractions explain heterogeneity in global soil carbon abundance and persistence. Global Change Biology 28:1178–1196.

Hicks Pries, C. 2022. Code for Tree and Fungal Identity Affect Soil Organic Matter Paper. Figshare. Software. https://doi.org/10.6084/m9.figshare.19624254.v1

Hicks Pries, C., N. Wurzburger, R. Lankau, O. Krol, G.A. Ingham, E. Legge, A. Fitch, J. Forrester. 2022. Soil organic matter, tree communities, and fungal communities across mycorrhizal gradients in the Eastern United States. Testing mechanisms of how mycorrhizal associations affect forest soil carbon and nitrogen cycling. ESS-DIVE repository. Dataset. https://doi.org/10.15485/1894515 accessed via https://data.ess-dive.lbl.gov/datasets/doi:10.15485/1894515 on 2022-10-18

Hicks Pries, C. E., J. A. Bird, C. Castanha, P.-J. Hatton, and M. S. Torn. 2017. Long term decomposition: the influence of litter type and soil horizon on retention of plant carbon and nitrogen in soils. Biogeochemistry 134:5–16.

Jackson, R. B., K. Lajtha, S. E. Crow, G. Hugelius, M. G. Kramer, and G. Piñeiro. 2017. The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. Annual Review of Ecology, Evolution, and Systematics 48:419–445.

Jo, I., S. Fei, C. M. Oswalt, G. M. Domke, and R. P. Phillips. 2019. Shifts in dominant tree mycorrhizal associations in response to anthropogenic impacts. Science Advances 5:eaav6358.

Kattge, J., G. Bönisch, S. Díaz, S. Lavorel, I. C. Prentice, P. Leadley, S. Tautenhahn, G. D. Werner, T. Aakala, and M. Abedi. 2020. TRY plant trait database–enhanced coverage and open access. Global change biology 26:119–188.

Keller, A. B., E. R. Brzostek, M. E. Craig, J. B. Fisher, and R. P. Phillips. 2021. Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. Ecology Letters 24:626–635.

Keller, A. B., and R. P. Phillips. 2019. Leaf litter decay rates differ between mycorrhizal groups in temperate, but not tropical, forests. New Phytologist 222:556–564.

Lang, A. K., F. V. Jevon, C. R. Vietorisz, M. P. Ayres, and J. Hatala Matthes. 2021. Fine roots and mycorrhizal fungi accelerate leaf litter decomposition in a northern hardwood forest regardless of dominant tree mycorrhizal associations. New Phytologist 230:316–326.

Langley, J. A., and B. A. Hungate. 2003. Mycorrhizal Controls on Belowground Litter Quality. Ecology 84:2302–2312.

Lankau, R. A., and D. P. Keymer. 2016. Ectomycorrhizal fungal richness declines towards the host species' range edge. Molecular Ecology 25:3224–3241.

Lavallee, J. M., J. L. Soong, and M. F. Cotrufo. 2020. Conceptualizing soil organic matter into particulate and mineral-assoc iated forms to address global change in the 21st century. Global Change Biology 26:261–273.

Lehmann, J., C. M. Hansel, C. Kaiser, M. Kleber, K. Maher, S. Manzoni, N. Nunan, M. Reichstein, J. P. Schimel, M. S. Torn, W. R. Wieder, and I. Kögel-Knabner. 2020. Persistence of soil organic carbon caused by functional complexity. Nature Geoscience 13:529–534.

Liang, C., W. Amelung, J. Lehmann, and M. Kästner. 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25:3578–3590.

Lilleskov, E. A., E. A. Hobbie, and T. R. Horton. 2011. Conservation of ectomycorrhizal fungi:

exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology 4:174–183.

Lin, G., M. E. Craig, I. Jo, X. Wang, D.-H. Zeng, and R. P. Phillips. 2022. Mycorrhizal associations of tree species influence soil nitrogen dynamics via effects on soil acid–base chemistry. Global Ecology and Biogeography 31:168–182.

Lin, G., M. L. McCormack, C. Ma, and D. Guo. 2017. Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. New Phytologist 213:1440–1451.

Lindahl, B. D., J. Kyaschenko, K. Varenius, K. E. Clemmensen, A. Dahlberg, E. Karltun, and J. Stendahl. 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. Ecology Letters 24:1341–1351.

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

Midgley, M. G., E. Brzostek, and R. P. Phillips. 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.

Morin, R. S., and A. M. Liebhold. 2015. Invasions by two non-native insects alter regional forest species composition and successional trajectories. Forest Ecology and Management 341:67–74.

National Atmospheric Deposition Program (NRSP-3). 2022. NADP Program Office, Wisconsin State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: an open annotation tool for parsing fungal community

datasets by ecological guild. Fungal Ecology 20:241–248.

Nilsson, R. H., K.-H. Larsson, A. F. S. Taylor, J. Bengtsson-Palme, T. S. Jeppesen, D. Schigel, P. Kennedy, K. Picard, F. O. Glöckner, L. Tedersoo, I. Saar, U. Kõljalg, and K. Abarenkov. 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264.

Nowacki, G. J., and M. D. Abrams. 2008. The Demise of Fire and "Mesophication" of Forests in the Eastern United States. BioScience 58:123–138.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2020. vegan: Community Ecology Package.

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

Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. New Phytologist 199:41–51.

R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasse, D. P., C. Rumpel, and M.-F. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil 269:341–356.

Rocci, K. S., J. M. Lavallee, C. E. Stewart, and M. F. Cotrufo. 2021. Soil organic carbon response to global environmental change depends on its distribution between mineral-associated and particulate organic matter: A meta-analysis. Science of The Total

Environment 793:148569.

Schmidt, M. W., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D. A. Manning, and others. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478:49–56.

See, C. R., A. B. Keller, S. E. Hobbie, P. G. Kennedy, P. K. Weber, and J. Pett-Ridge. 2022. Hyphae move matter and microbes to mineral microsites: Integrating the hyphosphere into conceptual models of soil organic matter stabilization. Global Change Biology 28:2527–2540.

See, C. R., M. L. McCormack, S. E. Hobbie, H. Flores-Moreno, W. L. Silver, and P. G. Kennedy. 2019. Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. Ecology Letters 22:946–953.

Sierra, C. A., M. Müller, H. Metzler, S. Manzoni, and S. E. Trumbore. 2017. The muddle of ages, turnover, transit, and residence times in the carbon cycle. Global Change Biology 23:1763–1773.

Siletti, C. E., C. A. Zeiner, and J. M. Bhatnagar. 2017. Distributions of fungal melanin across species and soils. Soil Biology and Biochemistry 113:285–293.

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

Sokol, N. W., S. E. Kuebbing, E. Karlsen-Ayala, and M. A. Bradford. 2019. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. New Phytologist 221:233–246.

Sollins, P., M. G. Kramer, C. Swanston, K. Lajtha, T. Filley, A. K. Aufdenkampe, R. Wagai, and R. D. Bowden. 2009. Sequential density fractionation across soils of contrasting

mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry 96:209–231.

Sollins, P., C. Swanston, M. Kleber, T. Filley, M. Kramer, S. Crow, B. A. Caldwell, K. Lajtha, and R. Bowden. 2006. Organic C and N stabilization in a forest soil: Evidence from sequential density fractionation. Soil Biology and Biochemistry 38:3313–3324.

Soong, J. L., C. Castanha, C. E. H. Pries, N. Ofiti, R. C. Porras, W. J. Riley, M. W. I. Schmidt, and M. S. Torn. 2021. Five years of whole-soil warming led to loss of subsoil carbon stocks and increased CO2 efflux. Science Advances 7:eabd1343.

Sulman, B. N., E. R. Brzostek, C. Medici, E. Shevliakova, D. N. L. Menge, and R. P. Phillips. 2017. Feedbacks between plant N demand and rhizosphere priming depend on type of mycorrhizal association. Ecology Letters 20:1043–1053.

Sulman, B. N., E. Shevliakova, E. R. Brzostek, S. N. Kivlin, S. Malyshev, D. N. Menge, and X. Zhang. 2019. Diverse mycorrhizal associations enhance terrestrial C storage in a global model. Global Biogeochemical Cycles.

Sun, T., S. E. Hobbie, B. Berg, H. Zhang, Q. Wang, Z. Wang, and S. Hättenschwiler. 2018. Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. Proceedings of the National Academy of Sciences 115:10392–10397.

Taylor, M. K., R. A. Lankau, and N. Wurzburger. 2016. Mycorrhizal associations of trees have different indirect effects on organic matter decomposition. Journal of Ecology 104:1576–1584.

Terrer, C., R. P. Phillips, B. A. Hungate, J. Rosende, J. Pett-Ridge, M. E. Craig, K. J. van Groenigen, T. F. Keenan, B. N. Sulman, B. D. Stocker, P. B. Reich, A. F. A. Pellegrini, E. Pendall, H. Zhang, R. D. Evans, Y. Carrillo, J. B. Fisher, K. Van Sundert, S. Vicca,

and R. B. Jackson. 2021. A trade-off between plant and soil carbon storage under elevated CO2. Nature 591:599–603.

Toju, H., A. S. Tanabe, S. Yamamoto, and H. Sato. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PloS one 7:e40863.

White, T. J., T. Bruns, S. Lee, and J. L. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315–322.

Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society (B) 73:3–36.

Wu, Y., M. Deng, J. Huang, S. Yang, L. Guo, L. Yang, J. Ahirwal, Z. Peng, W. Liu, and L. Liu. 2022. Global patterns in mycorrhizal mediation of soil carbon storage, stability, and nitrogen demand: A meta-analysis. Soil Biology and Biochemistry 166:108578.

Wurzburger, N., and E. N. J. Brookshire. 2017. Experimental evidence that mycorrhizal nitrogen strategies affect soil carbon. Ecology 98:1491–1497.

Zhu, K., M. L. McCormack, R. A. Lankau, J. F. Egan, and N. Wurzburger. 2018. Association of ectomycorrhizal trees with high carbon-to-nitrogen ratio soils across temperate forests is driven by smaller nitrogen not larger carbon stocks. Journal of Ecology 106:524–535.

Table 1. Soil and climate parameters of the four forest sites where we collected soils from stands (10-12 m radius) ranging in the proportion of tree basal area occupied by EcM-associated trees.

Site	Plots	Stand Age (year)	Soil Order	Soil Series	EcM range (%)	MAT/ MAP	Carbon (%)	Nitrogen (%)	Al+0.5Feox[1] (mg g^{-1} soil)	N dep[2] 2000, 2017 (kg N/ha/ y)
Chambeau River State Forest, Wisconsin (WI)	8 (8)[3]	80-100	Alfisol	Freeon Magnor	0-84	4.3°C/ 794 mm	1.9 ± 0.1	0.16 ± 0.03	5.4 ± 0.4	9.9, 10.9
Hanover, New Hampshire (NH)	11 (8)	>50	Inceptisol Spodosol	Pittstown Bernardstown	0-80	6°C/ 1092 mm	7.0 ± 0.5	0.33 ± 0.03	8.1 ± 0.7	8.8, 5.9
Allerton Park, Illinois (IL)	11 (11)	100	Mollisol	Sawmill	3-82	11.1°C/ 1020 mm	1.7 ± 0.07	0.14 ± 0.005	2.2 ± 0.1	10.8, 8.5
Chattahoochee National Forest, Georgia (GA)	8 (4)	80	Inceptisol Ultisol	Ashe Tusquittee Hayesville	32-87	13.4·°C/ 1750 mm	2.7 ± 0.3	0.12 ± 0.02	5.1 ± 0.7	9.6, 8.1

[1]Oxalate-extractable iron and aluminum

[2]Total inorganic nitrogen wet deposition data from the National Atmospheric Deposition Program (2022)

[3]The number of plots from which we were able to obtain fungal DNA sequences is in parentheses

Abbreviation: N dep[2] = N deposition[2]

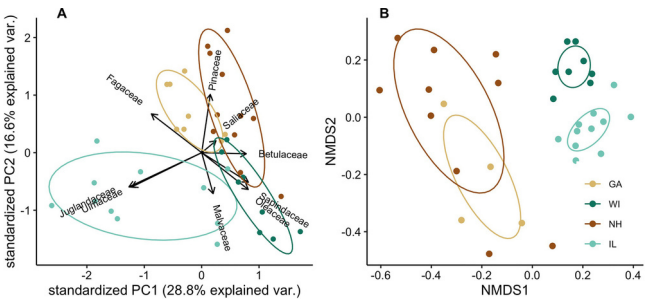
Figure 1. The communities of trees and EcM fungi varied among sites. (A) A PCA of the dominant tree families shows the forest stands in New Hampshire (NH) and Georgia (GA) had more similar tree communities by basal area than the forest stands in Wisconsin (WI) and Illinois (IL), particularly along the second principal component (PC2). PC2 was driven by the basal area of trees in the Pinaceae and Fagaceae families. (B) An NMDS of the compositional variation in EcM fungal communities (Bray-Curtis dissimilarity) shows the forest stands in NH and GA also have more similar EcM communities than forest stands in the WI and IL.

Figure 2. Only two sites (in GA and NH) demonstrated the expected relationship between soil organic matter characteristics and EcM basal area where the proportion of C and N in mineral-associated organic matter decreased (A,C) and the overall soil C:N increased (E) as EcM-associated trees became more dominant in the forest stand. In contrast, the second principal component (PC2) of the dominant plant family PCA was significantly related to soil organic matter characteristics. The proportion of C and N in MAOM decreased (B,D) and the bulk soil C:N increased (F) as the basal area of trees in the Pinaceae and Fagaceae became more dominant. Brown points represent forest stands in NH, tan in GA, dark green in WI, and light green in IL. The lines show the predictions from significant regressions ($\alpha<0.05$).

Figure 3. The proportion of C in MAOM (and N, not shown) decreased as the relative abundances of EcM genera with known peroxidase production capabilities increased. EcM with peroxidases included *Cortinarius*, *Lactarius*, *Piloderma*, and *Russula*. Brown points represent forest stands in NH, tan in GA, dark green in WI, and light green in IL. The black line shows the

prediction from a significant linear regression ($\alpha<0.05$) with the gray ribbon showing the 95% confidence interval.

Figure 4. Mechanisms by which plant (top) and fungal (bottom) traits associated with arbuscular mycorrhizae (AM) and ectomycorrhizae (EcM) can affect soil C:N ratios and the partitioning of organic matter among mineral-associated (MAOM) and particulate (POM) pools. For plant traits, EcM-associating trees tend to have low quality leaf (and sometimes root) litter that can lead to high soil C:N ratios and more organic matter being stored as POM. Recent evidence suggests that AM-associating trees may have higher rhizodeposition rates, which can encourage MAOM formation; however, rhizodeposition comparisons between AM and EcM trees are often idiosyncratic. For fungal traits, AM fungi acquire N by scavenging it from the environment while some EcM fungi can actively mine nitrogen from organic matter, which can induce competition with saprotrophs that may decrease decomposition and lead to more organic matter remaining as POM. Lastly, EcM fungi tend to produce more hyphal biomass and lower quality fungal necromass than AM fungi, which may also lead to reduced decomposition and more POM. The extent to which EcM mycorrhizae display the typical EcM traits listed here are taxa dependent. The pink background represents arbuscular mycorrhizal traits while the purple to blue color gradient represents the range of these traits found within EcM trees and fungi. Created with BioRender.com.



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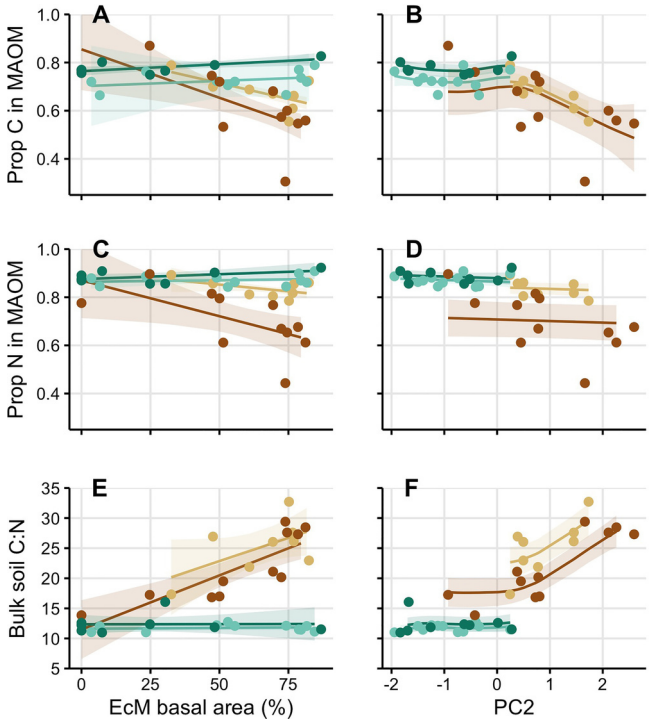

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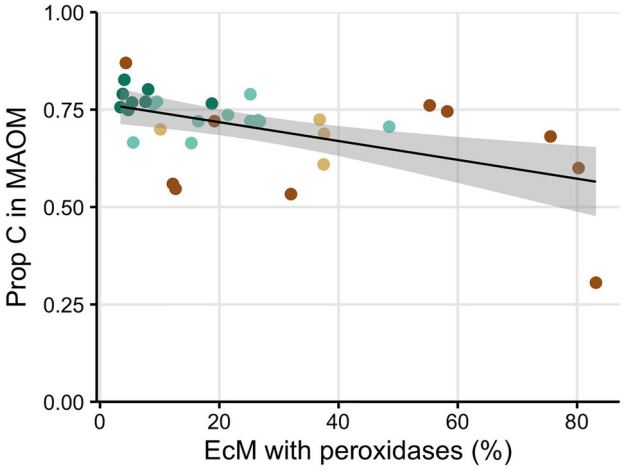

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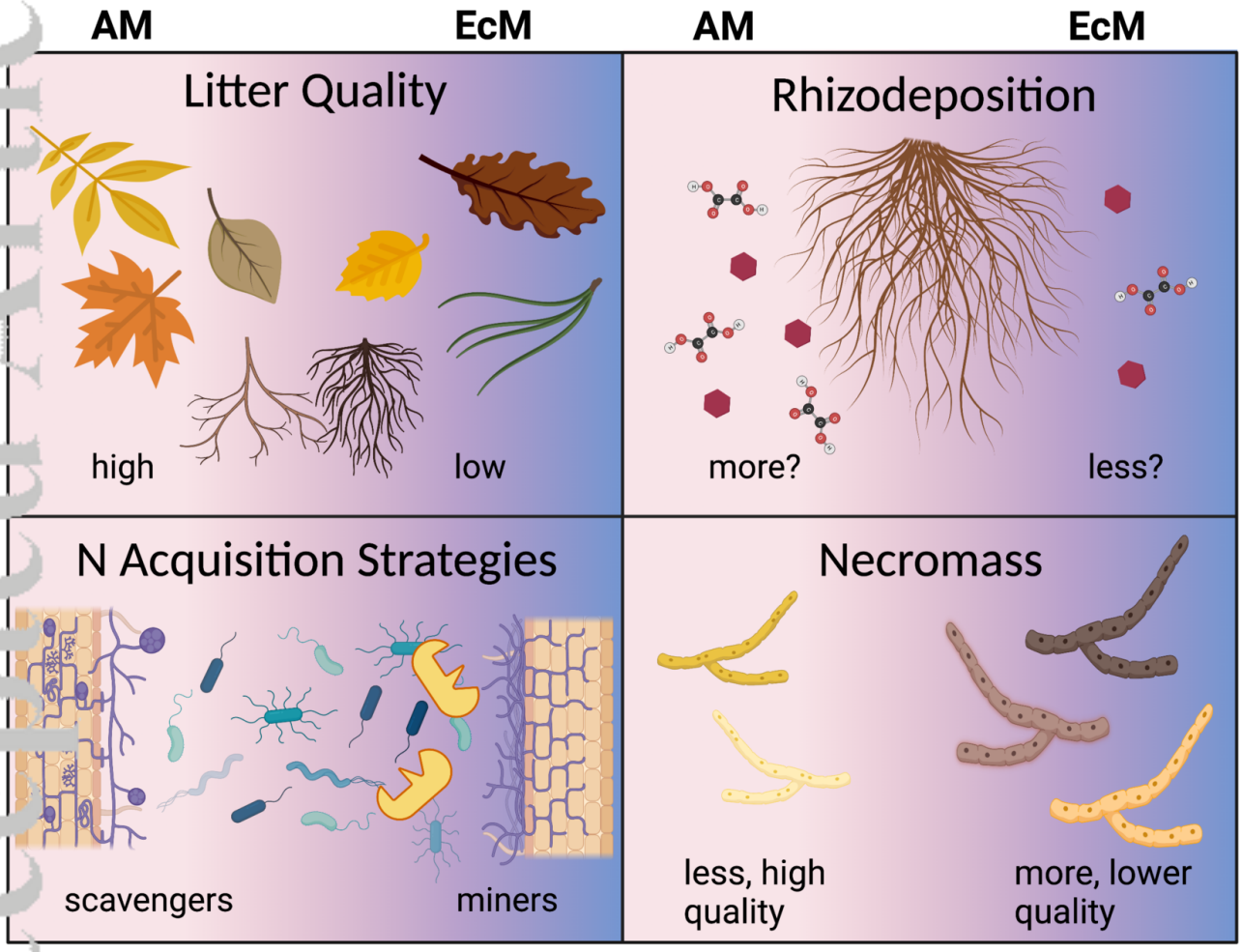

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