



Fungal Community, Not Substrate Quality, Drives Soil Microbial Function in Northeastern U.S. Temperate Forests

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Mycorrhizal fungi can affect soil organic matter cycling through several mechanisms including priming, nutrient competition, and direct enzyme production. Differences in nutrient foraging strategies between ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) fungi produce divergent belowground dynamics: where EcM can take up organic nitrogen and directly break down soil organic matter (SOM) by producing enzymes, AM fungi are limited to scavenging mineral N. EcM-associated tree species also have leaf litter with relatively higher ratios of carbon to nitrogen (C:N), and belowground saprotrophic communities more dominated by fungi. Consequently, free-living microbes in EcM-dominated soils should experience nitrogen limitation, with subsequent increases in enzyme production and decreased carbon use efficiency (CUE). However, the relative importance of the effects of substrate quality and fungal community composition on enzyme production and CUE are unclear. To assess this distinction, we sampled the organic horizon and 10 cm of the mineral horizon in northern temperate forest soils along a gradient of EcM dominance. We characterized fungal community composition by measuring EcM relative abundances from extracted fungal DNA and the fungal to bacterial (F:B) ratios from phospholipid fatty acid (PLFA) analysis. We assessed soil substrate quality as the soil C:N ratio. Soil microbial functions were measured as potential activities of five hydrolytic and two oxidative enzymes, and microbial CUE. We found that the fungal community, represented by either the F:B ratio, EcM relative abundance, or both, affected CUE and six measured enzyme activities, while the C:N ratio affected only oxidative and chitin-targeting extracellular enzyme activities. Our results highlight the use of EcM relative dominance as a predictor of soil microbial community composition and function independent of substrate quality.

Keywords: mycorrhizal fungi, forest soil, carbon use efficiency, enzymes, fungal:bacterial, carbon:nitrogen

INTRODUCTION

Global patterns show significant correlation between the mycorrhizal associations of canopy trees and soil carbon (C) and nitrogen (N) storage (Averill et al., 2014; Zhu et al., 2018; Lu and Hedin, 2019). Trees generally form symbioses with either arbuscular mycorrhizal (AM) or ectomycorrhizal (EcM) fungi where in return for increased soil nutrient uptake via mycorrhizal hyphae, plants exude a significant portion of their photo-assimilated carbon belowground to their mycorrhizal symbionts (Simard et al., 2003; Smith and Read, 2009; Ouimette et al., 2019). AM and EcM fungi have different nutrient acquisition strategies: while EcM fungi can more actively pursue soil organic N and compete with the surrounding soil microbial community, AM fungi are restricted to passively obtaining inorganic N after it has been liberated by other soil decomposers (Phillips et al., 2013). These strategies likely have divergent effects on the free-living bacterial and fungal saprotrophs thought to be primarily responsible for organic matter decomposition (Cheeke et al., 2017; Verbruggen et al., 2017; Lang et al., 2019). In order to understand the mycorrhizal controls on soil organic matter cycling observed at the global scale, we need to investigate how dominant mycorrhizal type (EcM versus AM) affects the function of soil microbial communities.

Mycorrhizal fungi can indirectly, and sometimes directly, affect soil extracellular enzyme activity (EEA), an important step in soil organic matter (SOM) decay. Many species of EcM fungi are able to produce enzymes that degrade plant cell walls, aiding in their ability to more directly access and compete for nutrients contained within organic matter (Cullings and Courty, 2009; Kohler et al., 2015). Given that mycorrhizal hyphal biomass can represent a third or more of the total microbial biomass in forest soils (Högberg and Högberg, 2002), the overall effect of EcM enzyme production should have a significant effect on organic matter decay (*sensu* Zak et al., 2019) and nutrient liberation even if their enzyme production rate is less than that of saprotrophic fungi (Talbot et al., 2008; Lindahl and Tunlid, 2015; Martin et al., 2016). Both EcM and AM fungi affect the surrounding soil microbial community by priming free-living saprotrophs with labile exudates to induce extracellular enzyme production and subsequent nutrient release (Hodge et al., 2001; Talbot et al., 2008; Kaiser et al., 2015; Gorka et al., 2019). This mycorrhizal-mediated priming mechanism can drastically increase or decrease depending on available soil nutrients, leading to shifts in the extracellular enzyme activity (EEA) of free-living microbes. Excess N, for example, has been shown to significantly decrease mycorrhizal-mediated exudation and EEA likely because plants decrease mycorrhizal access to photosynthate (Sinsabaugh et al., 2005; Högberg et al., 2010; Gorka et al., 2019).

This interaction between mycorrhizal priming, competition for nutrients, and differences in enzyme production is likely to affect microbial carbon use efficiency (CUE), the proportion of processed C that is incorporated into microbial biomass versus respired as CO₂. Specifically, microbes with access to an adequate supply of nutrients and easily assimilable substrates are expected to allocate more acquired C to biomass and less to CO₂ during respiration (Cotrufo et al., 2013;

Sinsabaugh et al., 2013; Geyer et al., 2016). These circumstances promoting elevated community CUE are hypothesized to be greater where mycorrhizal-mediated exudation rates are also higher. AM fungi are likely to have higher exudation rates relative to EcM fungi as they must induce free-living saprotrophic microbes to produce exoenzymes and liberate nutrients (Hodge et al., 2001; Kaiser et al., 2015). In EcM-dominated forests, competition for N between saprotrophic and mycorrhizal fungi has been associated with lower soil respiration and more C in the organic horizon, likely due to a suppression of saprotrophic microbial growth and biomass (Verbruggen et al., 2017; Zhu et al., 2018), potentially reducing CUE (Anthony et al., 2020). Further, increasing fungal:bacterial ratios in soils, which can be seen with increasing EcM dominance, have also been associated with less efficient community decomposition as saprotrophic fungi are less efficient than bacteria on average (Cheeke et al., 2017; Silva-Sánchez et al., 2019; Soares and Rousk, 2019). While C exudation by mycorrhizal fungi could promote a high microbial CUE in their saprotrophic neighbors, the activity of EcM fungi specifically may instead offset this positive effect by enhancing competition for N or by restricting exudation when N availability is high (Gorka et al., 2019; Hestrin et al., 2019).

Disentangling the effects of mycorrhizal fungi on the free-living soil microbial community is further complicated by differences in the quality of AM and EcM tree leaf litter, an important C and nutrient source for saprotrophic microbes. AM-associated trees usually have high quality, lower carbon:nitrogen (C:N) leaf litter and EcM-associated trees have lower quality, higher C:N leaf litter (Cornelissen et al., 2001; Sun et al., 2018). The quality of organic substrates may affect CUE and enzyme production since low-quality substrates contain fewer nutrients and are relatively slow to break down (Saiya-Corka et al., 2002; Cotrufo et al., 2013). For example, litter with a low C:N degrades quickly and has been found to promote more efficient microbial growth or faster litter decomposition, but without a consistent increase in enzyme production (Sinsabaugh et al., 2002; Frey et al., 2013; Lashermes et al., 2016). These findings suggest that substrate quality, CUE, and enzyme production are likely linked to interactions between mycorrhizal fungi and their free-living soil microbial community (Allison, 2012b). Yet, the degree to which substrate quality and dominant mycorrhizal type influence soil microbial function remains poorly described.

In order to understand how mycorrhizal associations affect soil microbial function, we assessed two hypothesized drivers of mycorrhizal control, fungal community composition and substrate quality, and related them to soil microbial functions—community CUE and potential extracellular enzyme activities. We measured these potential drivers and functions along a gradient of EcM-associated tree basal area (subsequently referred to as EcM basal area) in northern temperate forests. We characterized (i) changes in fungal community composition (assessed here as EcM relative abundance, AM biomass, and fungal:bacterial ratios) and (ii) substrate quality (assessed as soil C:N) with soil horizon (organic and top 10 cm of mineral soil). We tested three hypotheses: (i) With increasing EcM basal area,

soil C:N, EcM relative abundance, and fungal to bacterial (F:B) would all increase, while AM biomass would decrease; (ii) the relative importance of substrate quality on extracellular enzyme activities would decrease from the organic to the mineral soil, because the organic horizon contains more complex organic substrates requiring enzymatic degradation; and (iii) the fungal community composition would be a more important driver of CUE than substrate quality. Specifically, we predicted that the CUE of the free-living microbial community would decrease as EcM became more dominant due to EcM-driven competition for N and subsequent saprotrophic growth limitation. We further predicted a decline in free-living community CUE where EcM dominate, because we expected a concomitant increase in overall fungal dominance (i.e., increasing F:B), and saprotrophic fungi are less efficient per unit substrate than bacteria.

MATERIALS AND METHODS

Study Sites

We collected soils across gradients of EcM basal area at two sites, one in Norwich, Vermont (43.72°N, 72.35°W) and one in Hanover, New Hampshire (43.72°N, 72.16°W) on 22 May 2019, during the early growing season. These sites are classified as mixed hardwood forest on inceptisols. We collected soils from 10, 10 m radius plots across a gradient of EcM basal area (**Supplementary Table S1**). Within each plot, we measured the diameter at breast height (1.4 m) of all trees greater than 10 cm in diameter and identified each tree to species. We assigned all trees as EcM or AM based on their family (**Supplementary Table S1**). The basal area of Salicaceae was assumed to be 50% EcM, and only one species from our plots, *Populus tremuloides*, was in this family. EcM species in the lowest EcM basal area plot were represented mostly by *Betula papyrifera*, while mid-range EcM basal area plots included an increasing presence of *Fagus grandifolia*, *P. tremuloides*, and *Quercus Rubra*. The highest EcM basal area plots were dominated by *Tsuga canadensis*.

Soil Collection and Processing

At each site, we collected soil cores of the organic horizon, which ranged in thickness from 4 to 12 cm, and the corresponding top 10 cm of mineral soil. We collected soils cores from two spots at the center of our circular plots and combined those cores for analysis. To collect the cores, we first cut out and set aside the O horizon within the footprint of a 5 cm diameter stainless steel corer, which we then used to collect the mineral soil. Samples were transported back to the lab on ice and stored at 4°C before further processing. Organic horizon samples were root picked and thoroughly mixed while mineral samples were sieved through a 2 mm mesh. Subsamples were dried overnight at 100°C, ground, and later analyzed for percent soil C and N using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, United States). Soil pH was measured with a 1:1 ratio of soil to deionized water from the thawed organic horizon and mineral soils using a benchtop pH meter (Mettler Toledo, Columbus, OH, United States).

Extracellular Enzyme Assays

We performed fluorometric and colorimetric assays of EEA using methods modified from German et al. (2011) and Allison (2012a). We measured EEA for hydrolases: β -glucosidase (BG; glucose from cellulose), cellobiohydrolase (CB; disaccharides from cellulose), *N*-acetyl-glucosaminidase (NAG; chitin), Leucine-aminopeptidase (LAP; amino acids from proteins), and acid phosphatase (AP; phosphate ions from organic compounds); and two oxidases: phenol oxidase (PO) and peroxidase (PER). In brief, soil subsamples (1 g) from thawed soils were homogenized using an immersion blender with 125 ml of deionized water, to ensure capturing *in situ* soil pH. 200 μ l of sample homogenate was pipetted into 96-well plates with 50 μ l of each 4-Methylumbelliferone labeled-fluorogenic substrate in replicates of four and incubated in the dark at 12°C (Koch et al., 2007). Fluorescence was measured on a microplate spectrophotometer (365 nm excitation, 450 nm emission) for hydrolases after addition of 15 μ l of 1 M NaOH. For PO and PER, the samples were mixed with 50 μ l L-3,4-dihydroxyphenylalanine (L-DOPA) or 50 μ l L-DOPA and 20 μ l hydrogen peroxide, respectively, incubated in the dark at 12°C for 18 h, and then their absorbance (460 nm) was measured on the microplate reader. We used a principle component analysis (PCA) to group enzymes for subsequent statistical analyses based on relatedness across samples (**Supplementary Figure S1**). BG and CB were summed to create a hydrolytic activity metric as these enzymes target plant derived, carbon-based molecules (Emilsson et al., 2016), and LAP and AP were grouped as nutrient-targeting enzymes given their similar PCA loadings (**Supplementary Figure S1** and **Supplementary Table S2**).

Microbial Carbon Use Efficiency

We measured CUE of the organic and mineral soil microbial communities following an ^{18}O tracing method, where CUE is estimated based on ^{18}O -water incorporation into microbial DNA (Spohn et al., 2016; Geyer et al., 2019). Microbial communities were first acclimated by pre-incubating samples for 5 days at 12°C. We added a dilution of labeled ^{18}O -water and ^{16}O -water to 0.3 g of soil from the same sample in separate 2 ml microcentrifuge tubes. Dilutions of ^{18}O -water were determined by optimizing for a 20 atom% label in each sample without exceeding the average water holding capacity of the mineral and organic soils (see Data Availability Statement section for link to data repository which contains spreadsheet “18O_CUEcalcs.SCxTS.2019.xls,” tab name “prep calcs,” for full dilution calculations). In short, we aimed for soils to be moist, but not saturated to avoid anaerobic conditions. We then inserted the microcentrifuge tubes into 50 ml glass incubation bottles, which were sealed and flushed with CO_2 -free air prior to a 48 h incubation at 12°C. Following incubation, we measured 10 ml of headspace CO_2 using an EGM-5 CO_2 gas analyzer (PP-Systems, Amesbury, MA, United States). We removed microcentrifuge tubes from bottles and extracted microbial DNA following modified methods, where the entire supernatant was transferred in order to extract total DNA as opposed to a subsample, and using materials from the Qiagen DNeasy Powersoil extraction

protocol and kit. An important modification step is to use all the supernatant in every step (instead of a subset), as the final calculations are based on the amount of ^{18}O incorporated in the DNA. We added 70 μl of the final extractions to silver capsules (5×3.5 mm) along with 100 μl of 10 ng ml^{-1} salmon sperm to raise the oxygen to detection level. We then dried samples at 60°C overnight and sent the capsules in a 96-well plate to the UC Davis Stable Isotope facility for ^{18}O analysis on an elemental PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany) and interfaced to an Isoprime VisION (Isoprime Ltd., Stockport, United Kingdom, a unit of Elementar Analysensysteme GmbH, Hanau, Germany).

In parallel, we analyzed total organic carbon (TOC) in microbial cells by performing a chloroform fumigation and subsequent TOC extraction on duplicate 15 mg thawed soil samples (Vance et al., 1987). One set of samples was fumigated with chloroform overnight to lyse microbial cells. TOC was subsequently extracted from both fumigated and duplicate non-fumigated soil using 0.5 M KCL. The extracts were diluted to a ratio of 1:4 for TOC analysis on a Sievers M5310 C Laboratory TOC analyzer (SUEZ, Paramous, NJ, United States).

DNA Extraction and Bioinformatics

Fungal DNA was extracted from organic horizon and mineral soils using the Quiagen PowerSoil Kit (Quiagen Science Inc., Germantown, MD, United States). DNA extracts were sent to the University of Wisconsin-Madison Biotechnology Center where they were prepared similarly to the process detailed in Illumina's 16s Metagenomic Sequencing Library Preparation Protocol, Part # 15044223 Rev. B (Illumina Inc., San Diego, CA, United States). The ITS region was amplified using the forward primers ITS (CTTGGTCATTTAGAGGAAGTAA) and reverse primer ITS (TCCTCCGCTTATTGATATGC) (Gardes and Bruns, 1993). Duplicate PCR reactions were conducted under the conditions outlined in Caporaso et al. (2011). PCR products were cleaned using an AxyPrep Mag PCR clean-up kit (Axygen Biosciences, Union City, CA, United States). Quality and quantity of the cleaned products were assessed using an Agilent DNA 1000 kit (Agilent Technologies, Santa Clara, CA, United States) and Qubit dsDNA HS Assay Kit (ThermoFisher Scientific), respectively. Libraries were pooled in an equimolar fashion prior to sequencing, and paired-end, 300 bp sequencing was performed using Illumina MiSeq (v3). Sequences were processed using R software (R Core Team, 2019, version 3.6.1), with the Dada2 package and following the Dada2 ITS-specific pipeline (Callahan et al., 2016). The ITS1 region was extracted using itsxpress for downstream community analysis (Rivers et al., 2018). Unique amplicon sequence variants (ASVs) were identified to the lowest confident taxonomic level using a Naïve Bayesian Classifier from the UNITE ITS database (Wang et al., 2007). Singletons and non-fungal sequences were removed and samples were rarefied to a sequencing depth of 16,803 using the "rrarefy" function in R ("vegan" package; Oksanen et al., 2018). Thereafter, ASV relative abundance was calculated for each sample. EcM ASVs were identified using the fungal functional group assignment software, FUNGuild (Nguyen et al., 2016). The relative abundance of EcM fungi within

the sequenced fungal community (referred to as EcM relative abundance hereafter) was calculated as the sum of the relative abundance of EcM ASVs divided by the sum of all relative abundance ASVs. EcM richness was calculated as the number of EcM ASVs per sample. The corresponding ASV dataset (filename "SCxTS_ASV_taxonomy.csv") prior to rarefaction can be accessed at the location specified in "Data Access."

Phospholipid Fatty Acid Analysis

We estimated the biomass of AM fungi and the ratio of bacterial relative abundance to fungal relative abundance (F:B) from soil samples using phospholipid fatty acid (PLFA) analysis by gas chromatography mass spectroscopy (Microbial ID, Inc., Newark, DE, United States). Thawed subsamples were processed using standard methods (Buyer and Sasser, 2012). Peaks for AM fungi (16:1 ω 5) and saprophytic fungi (18:2 ω 6) were identified using the Sherlock System (MIDI, Inc., Newark, DE, United States). Though no method of analyzing fungal biomass and dominance in soil is without limitations (Strickland and Rousk, 2010), we used these metrics to reflect the relative composition of the microbial community along our mycorrhizal gradient.

Statistical Analysis

All statistical analyses were performed using R software (R Core Team, 2019, version 3.6.1). We used linear mixed modeling in lme4 (Bates et al., 2015) to predict C:N and fungal community metric responses to EcM basal area, as well as microbial functional responses (potential enzyme activities and CUE) to C:N and fungal community metrics. We used a PCA to calculate and visualize the importance of community metrics: EcM relative abundance explained the most variation along axis 1, while the F:B ratio explained the most variation along axis 2 (**Supplementary Figure S1** and **Supplementary Table S4**). Thus, we included EcM relative abundance and F:B as predictors in the linear mixed models for microbial functions. Specifically, linear mixed model predictors included the main effects of C:N ratio, EcM relative abundance, and the F:B ratio, and their interactions with soil horizon. We checked predictors for autocorrelation by calculating their correlation coefficients: all coefficients were less than 0.54. Predictor variables were scaled in order to compare effect sizes among estimates. Given that pH was highly correlated with several of our focal predictors in the EcM basal area models, pH was omitted from microbial function models (**Supplementary Table S3**). While we recognize that pH is an important control on microbial community composition, it was not part of our hypotheses; our intention was to investigate how community composition affects microbial function, which is downstream of the pH effect. We included sample core, because the mineral and organic horizon samples come from the same core at each plot, as a random effect in each model. We performed model selection by sequentially removing interactions and then predictors with the least significant *p*-value and verifying that the AIC value for the newest model did not increase relative to the previous model (Bolker et al., 2009). We verified model variance homogeneity by plotting model residuals by each of the model predictors at each step of the model selection process, and model normality by plotting model residuals in

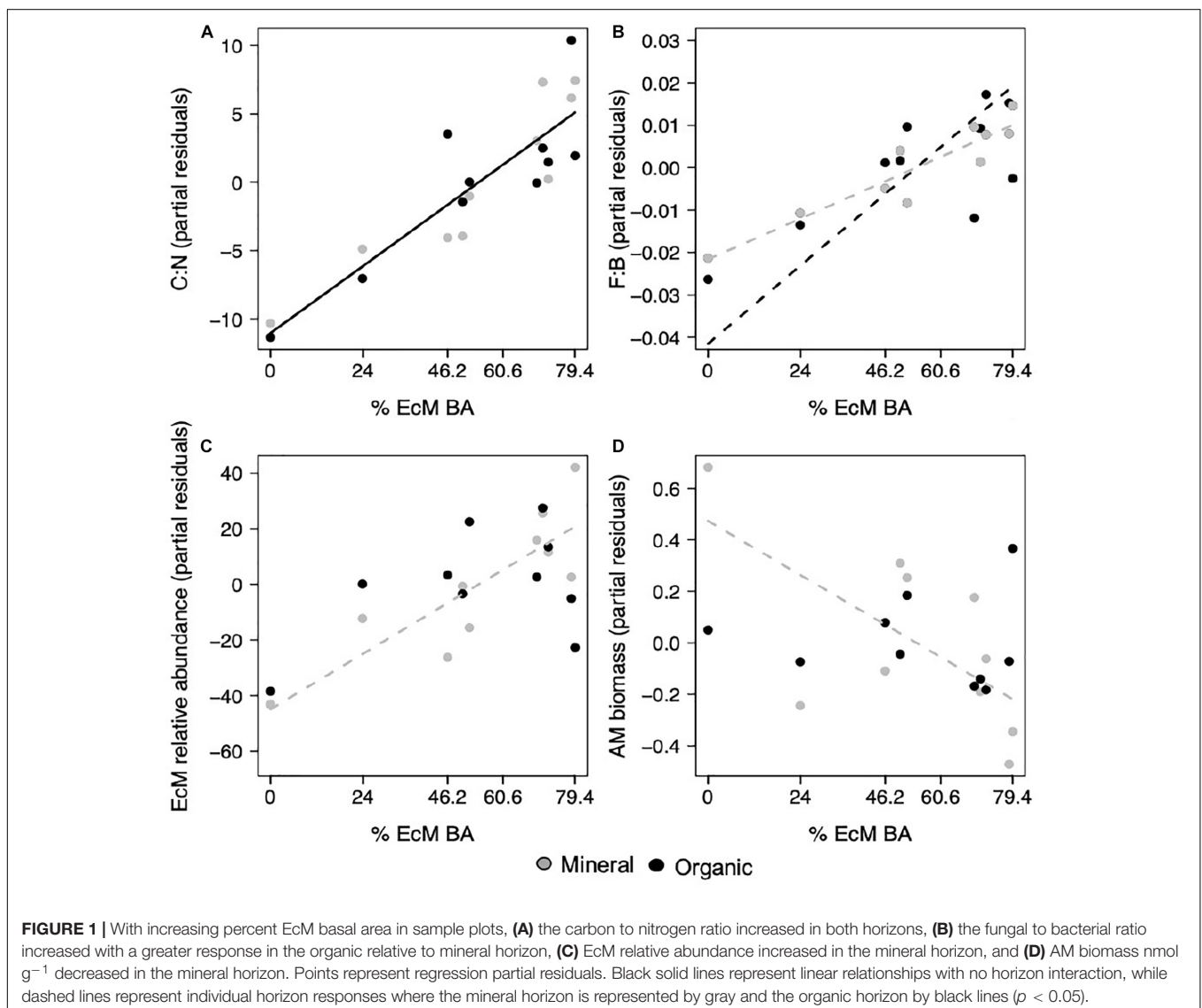
a histogram. We also analyzed model fit by calculating the marginal and conditional coefficient of variation using the function `R.squaredGLMM` (Supplementary Table S5; Barton, 2009). We visualized significant responses by plotting model partial residuals for the response variable by the raw predictor values. Plots of the raw response variables by the predictors are available in Supplementary Figures S3–S6.

RESULTS

Substrate Quality and Microbial Community Composition Along the EcM Basal Area Gradient

With increasing EcM basal area, which ranged from 0 to 79.4%, we found that the soil C:N ratio increased in both the organic, ranging from 12.9 to 32.4, and mineral horizon, ranging from

13.9 to 29.4 ($p = 0.001$; Figure 1A and Supplementary Table S3). However, patterns in fungal community composition were less clear. The F:B ratio increased with EcM basal area, but this pattern was stronger in the organic, ranging from 0.041 to 0.123, relative to mineral horizon, ranging from 0.036 to 0.077 ($p < 0.001$, $p = 0.026$; Figure 1B and Supplementary Table S3). We verified that F:B was not driven by changes in just fungal or bacterial relative abundance, and was positively correlated with the relative fungal abundance and negatively correlated with relative bacterial abundance ($r = 0.89$, $p < 0.001$; $r = -0.83$, $p < 0.001$). We also found that F:B and EcM relative abundance were not significantly correlated ($r = 0.28$, $p = 0.231$). EcM relative abundance, which ranged from 5.20 to 85.94 in the organic and from 2.96 to 75.38 in the mineral horizon, increased with EcM basal area in the mineral, but not organic horizon ($p = 0.019$; Figure 1C and Supplementary Table S3). AM biomass (nmol g^{-1}), which ranged from 24.31 to 40.53 in the organic and from 4.12 to 12.57 in the mineral, decreased in the



mineral but not in the organic horizon ($p = 0.051$; **Figure 1D** and **Supplementary Table S3**). AM biomass was included as an estimate of AM abundance because the ITS sequencing approach we employed is biased against AM fungi. Fungal richness was not related to EcM basal area (**Supplementary Table S3**).

The Effect of Fungal Community on Microbial Function

Most extracellular enzyme activities were related to either F:B or EcM relative abundance, but not both. Specifically, several enzymes had opposing relationships with F:B. PER, an enzyme class that depolymerizes lignin polymers, decreased significantly with increasing F:B in both horizons ($p = 0.007$; **Figure 2B** and **Table 1**). In contrast, AP + LAP, enzymes that target phosphorus-containing organic compounds and peptide chains, respectively, as well as PO, which oxidizes lignin monomers, increased significantly with increasing F:B in the mineral horizon ($p = 0.007$, $p = 0.019$, respectively; **Figures 2C,D**) and marginally in the organic horizon ($p = 0.093$, $p = 0.092$, respectively). Enzymes targeting simple C compounds (BG + CB) and chitin-bound N (NAG) were not affected by the F:B ratio. In contrast EcM relative abundance affected only simple C-degrading enzyme (BG + CB) activities ($p = 0.024$, respectively; **Figure 3B**).

Increases in F:B were associated with lower microbial CUE in the mineral horizon ($p = 0.012$; **Table 1**), but did not affect CUE in the organic horizon (**Figure 2A**). In comparison, CUE increased with EcM relative abundance across both horizons ($p = 0.016$; **Figure 3A** and **Table 1**). The effect size for F:B was 2.8 times the effect size for EcM relative abundance.

The Effect of Substrate Quality on Microbial Function

Microbial functions were less affected by the soil C:N ratio, with significant effects only observed in the organic horizon. Only two enzymes, PER ($p = 0.021$; **Figure 4A**) and NAG ($p = 0.010$; **Figure 4B**), increased in activity as soil C:N increased. Microbial CUE was not affected by the soil C:N ratio (**Table 1**).

DISCUSSION

We compared the effects of fungal community composition and substrate quality on soil microbial function across a gradient of EcM tree basal area. We demonstrated distinct microbial functional signatures of soils dominated by EcM versus AM trees, and we identified fungal community structure, rather than substrate quality, as the major driver of divergent microbial functions.

Substrate Quality and Microbial Community Composition Change With EcM Basal Area

Our first hypothesis, as to how substrate quality and fungal community composition would change along a gradient of EcM basal tree area was supported in the mineral horizon for AM biomass and EcM relative abundance, and across both horizons

TABLE 1 | Mean effect sizes \pm standard error (SE), t -values, degrees of freedom (df), and p -values for microbial function linear mixed effects model outputs.

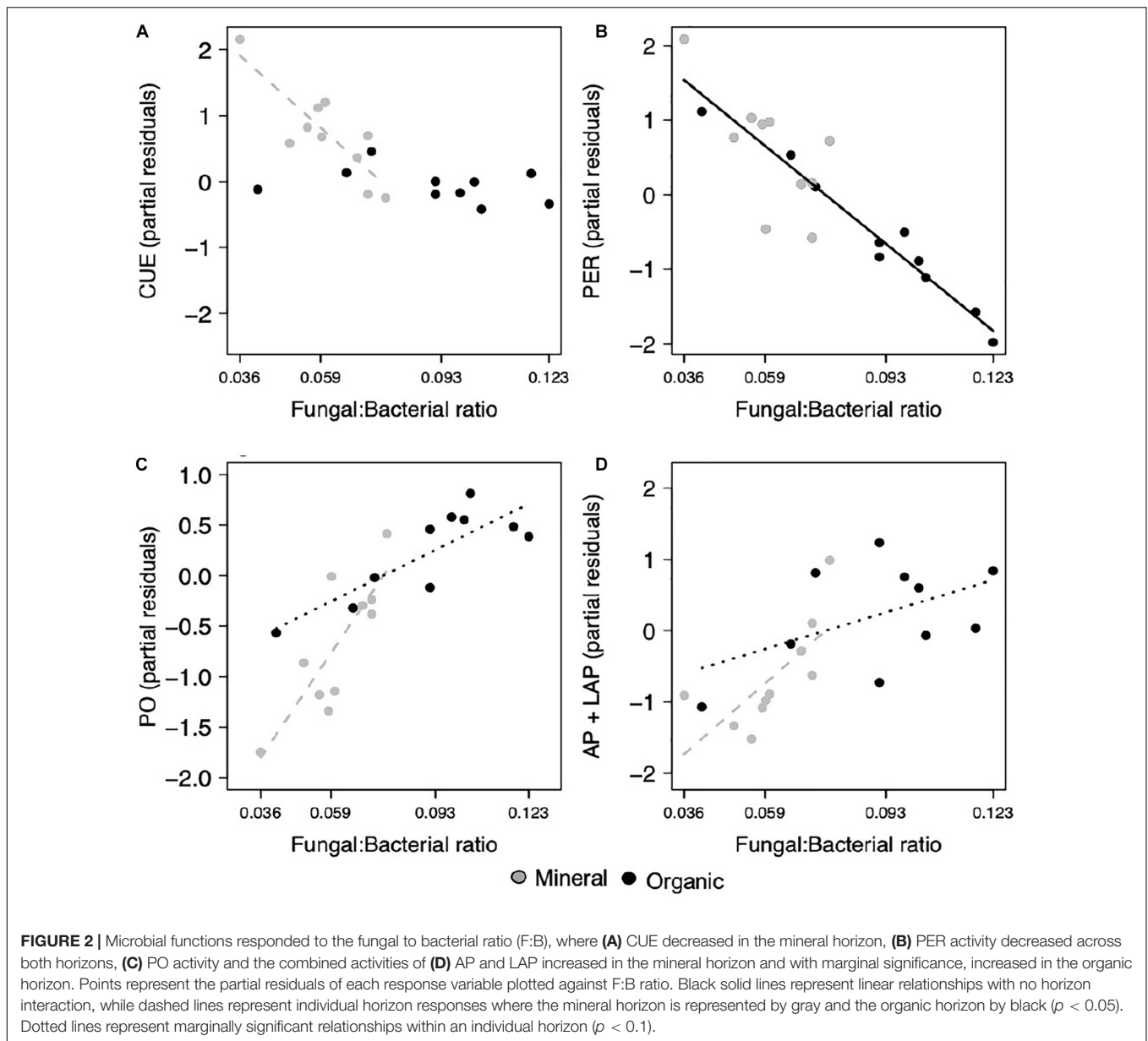
Response	Predictor	Mean effect \pm				p -value
		[SE]	t	df		
CUE	Intercept	-2.52 ± 0.33	-7.66	12.09	<0.001	
	EcM.RA	0.43 ± 0.16	2.73	14.65	0.016	
	Horizon	0.86 ± 0.40	2.17	13.11	0.049	
	F:B*Mineral	-1.18 ± 0.44	-2.67	12.17	0.02	
	F:B*Organic	-0.09 ± 0.20	-0.44	13.25	0.67	
BG + CB	Intercept	10.14 ± 0.19	53.3	10.68	<0.001	
	CN	0.05 ± 0.21	0.26	15	0.8	
	EcM.RA	0.37 ± 0.14	2.58	12.35	0.024	
	Horizon	0.52 ± 0.16	3.33	6.93	0.013	
	CN*Horizon	0.29 ± 0.17	1.68	6.97	0.137	
PER	Intercept	1.04 ± 0.27	3.86	12.62	0.002	
	F:B	-0.95 ± 0.29	-3.28	11.46	0.007	
	Horizon	1.58 ± 0.41	3.86	14.99	0.002	
	CN*Mineral	0.33 ± 0.23	1.45	14.35	0.169	
	CN*Organic	0.85 ± 0.29	2.95	13.98	0.011	
PO	Intercept	1.73 ± 0.33	5.26	14.24	<0.001	
	Horizon	-1.18 ± 0.16	-3.05	15.76	0.008	
	F:B*Mineral	1.11 ± 0.42	2.67	15.48	0.017	
	F:B*Organic	0.37 ± 0.21	1.8	15.48	0.092	
	NAG	Intercept	10.50 ± 0.36	28.94	13.91	<0.001
NAG	Horizon	1.18 ± 0.49	2.24	7.24	0.045	
	CN*Mineral	-1.09 ± 0.61	-1.8	13.97	0.094	
	CN*Organic	1.17 ± 0.38	3.09	13.95	0.008	
	EcM.RA*Mineral	1.12 ± 0.60	1.86	13.97	0.083	
	EcM.RA*Organic	-0.03 ± 0.38	-0.08	13.92	0.936	
	AP + LAP	Intercept	10.09 ± 0.32	31.36	16	<0.001
	Horizon	-0.48 ± 0.40	-1.2	16	0.249	
AP + LAP	F:B*Mineral	1.06 ± 0.42	2.57	16	0.021	
	F:B*Organic	0.37 ± 0.21	1.79	16	0.093	

Bold values designate significant p -values (<0.05).

for C:N and the F:B ratio. The increase in soil C:N was likely due in part to the relatively lower quality (i.e., higher C:N) leaf litter of the dominant EcM-associated tree species (Phillips et al., 2013; Sun et al., 2018). Concurrently, EcM fungi can actively increase soil C:N ratios by their ability to mine and take up organic N (Averill et al., 2014; Sterkenburg et al., 2018; Zhu et al., 2018).

The increase in EcM relative abundance and corresponding decrease in AM biomass in the mineral horizon confirm that the basal area of trees reflects the abundance of their associated mycorrhizal symbionts. However, this relationship may have been weaker in the organic horizon due to potential vertical stratification of mycorrhizal-associated root tips and foraging extramatrical mycelium (Genney et al., 2006). For instance, studies from Boreal systems have shown vertical separation of EcM and saprotrophic fungi, indicating the importance analyzing both mineral and organic soil horizons in order to understand EcM fungal effects (Rosling et al., 2003; Lindahl et al., 2007).

The F:B ratio increased with EcM basal area across both horizons, suggesting that soil below EcM-associated trees harbors

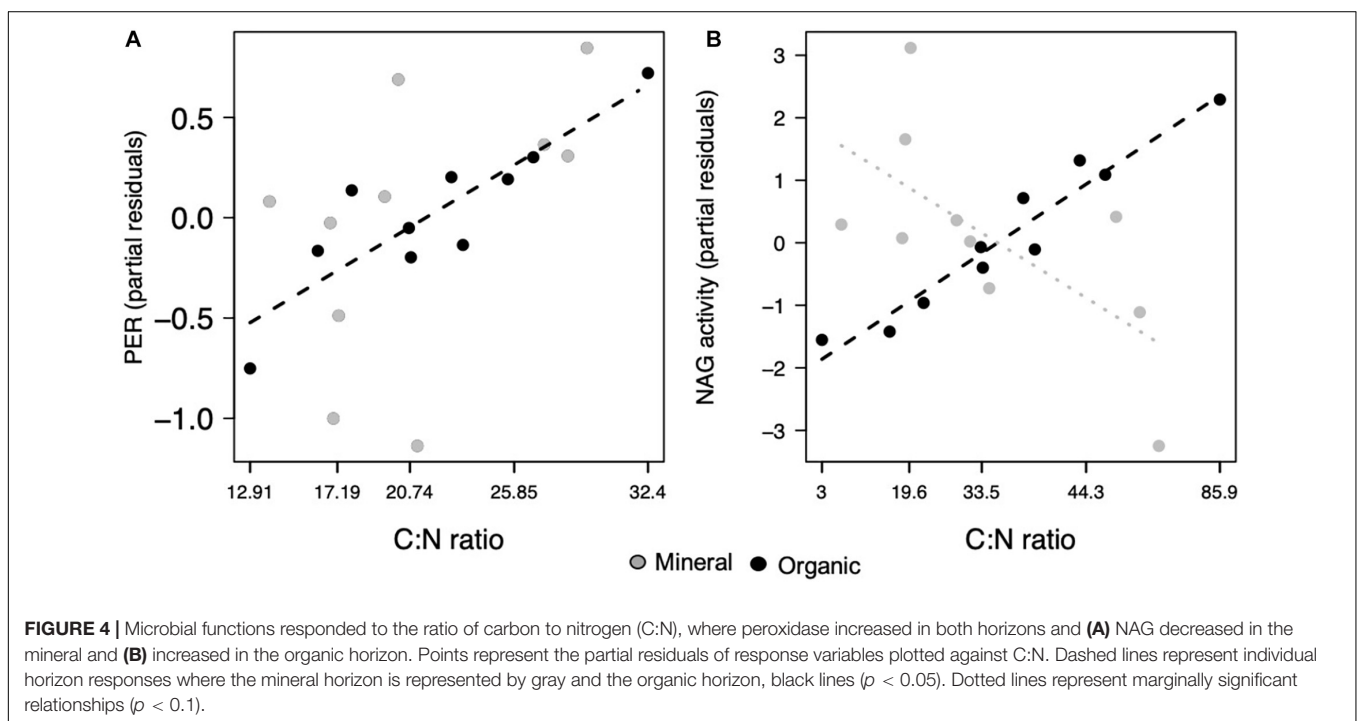
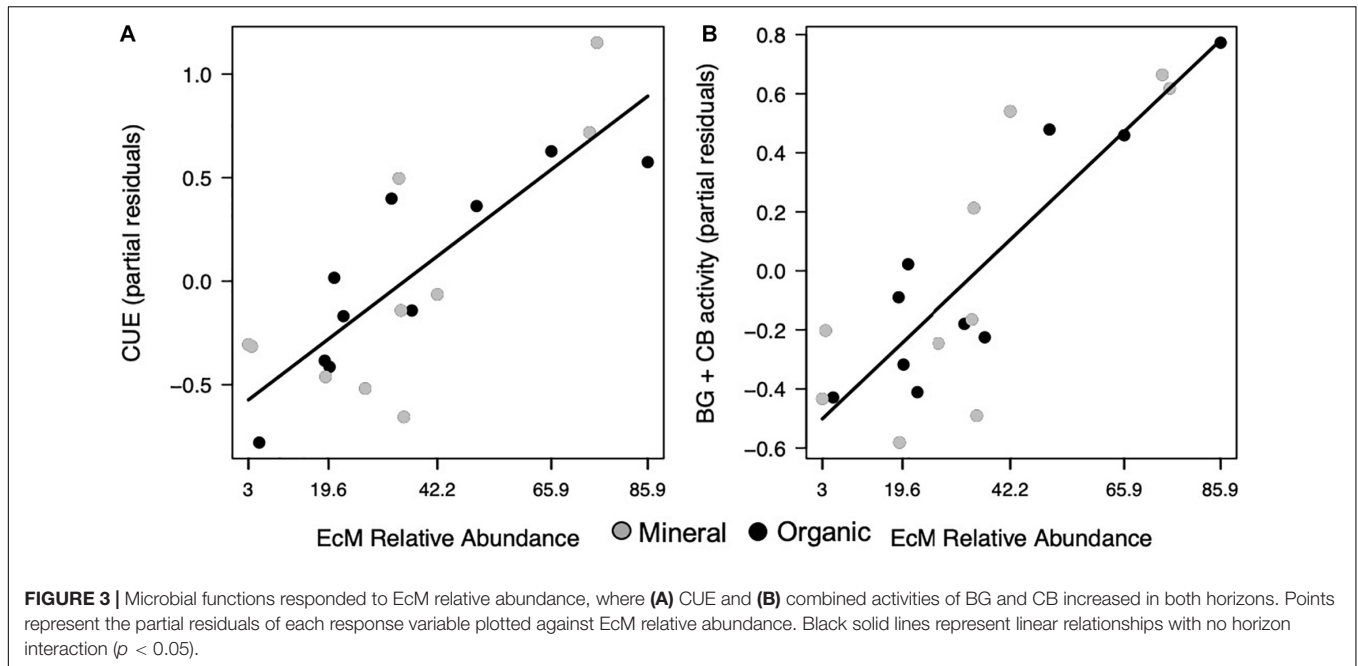


a more fungal-dominated community than soil below AM-associated trees (Cheeke et al., 2017), as the F:B ratio was strongly and positively correlated with fungal relative abundance. Increasing saprotrophic fungal dominance could have been affected by the concurrent decreasing litter quality along the EcM gradient. Fungi have a lower N requirement than bacteria and can use the relatively low-N substrates available in EcM-dominated plots, giving them a competitive advantage despite having lower growth efficiency than bacteria (Fierer et al., 2009; Güsewell and Gessner, 2009; Silva-Sánchez et al., 2019). It is also possible that this increasing F:B signature was affected by elevated production of EcM mycelium and biomass, as the PLFA analysis does not distinguish between EcM and saprotrophic fungi. However, the non-significant correlation between F:B and EcM relative abundance supports

our interpretation of the effects of F:B as being driven primarily by saprotrophic fungi.

Effect of Substrate Quality Versus Fungal Community Composition on Microbial Function

Our analysis showed that fungal community composition is a more important driver of microbial function along a gradient of EcM basal area than substrate quality. Although changes in the C:N ratio were associated with increases in two enzymes (NAG and PER), the majority of soil microbial functions exhibited stronger relationships with fungal community metrics. The positive response of NAG to increasing C:N in the organic horizon may reflect the need to liberate N from organic sources,



such as chitin in fungal necromass (Gorka et al., 2019; Schnecker et al., 2019), while PER activity likely increased with C:N as the microbial community degraded more complex substrates such as lignin (Rinkes et al., 2013; Lin et al., 2017), which is generally enriched in EcM tree litter relative to that of AM trees (Sun et al., 2018). However, PER activity also responded negatively to an increasing F:B ratio across both soil horizons, with a greater effect size and significance relative to the effect of C:N. Therefore, although PER production is sensitive to

local substrate quality, microbial community structure is of greater importance.

Fungal Community Components Differentially Affect EEA

Most extracellular enzyme activities responded to either the F:B ratio or the relative abundance of EcM fungi, but not both, suggesting EcM fungi and saprotrophic fungi control

specific groups of enzymes. Specifically, the combined activities of BG + CB increased with EcM relative abundance, but not the F:B ratio, suggesting that this particular suite of enzymes, which degrade plant starch and cellulose, is regulated directly by EcM fungi in these soils rather than both EcM and saprotrophic fungi. This group of enzymes is likely directly produced by EcM fungi, induced by EcM priming, or a combination of both mechanisms (Kotroczo et al., 2014; Kohler et al., 2015). The lack of relationship between EcM relative abundance and the oxidative enzymes PER and PO is not surprising; during evolution from saprotrophic ancestors, most EcM taxa lost the ability to produce oxidative enzymes responsible for lignin degradation (Martin et al., 2016).

Differences in microbial community composition could explain the increase in PO activity and decrease in PER activity with higher F:B. Fungal taxa that are proficient in producing POs (e.g., laccase), but lack the ability to produce PERs were likely present in these soils (Sinsabaugh, 2010; Martin et al., 2016). Moreover, declining PER activity with F:B suggests that PER production at our site may be dominated by bacteria. Recent research has found that bacteria produce a family of lignin-degrading PERs that are structurally unrelated to fungal PERs (de Gonzalo et al., 2016). Thus, as F:B increased, relatively fewer bacteria may have resulted in lower PER activity.

The combined activities of AP + LAP, which target phosphorus-containing organic compounds and peptide chains, respectively, also increased with the F:B ratio, and did not respond to substrate quality. These results indicate that nutrient-targeting enzyme production is not necessarily induced by the nitrogen availability in soil (Waring, 2013). Instead, saprotrophic fungi appear to be important for degrading N and P containing organic compounds (Talbot et al., 2015). Further, EcM-dominated ecosystems are hypothesized to have developed more fungal-dominated free living communities because their relatively greater enzymatic capacities are needed to break down the lower quality leaf litter (Cheeke et al., 2017). Given that both F:B and C:N increased along the EcM basal area gradient, it is possible that these two seemingly different mechanisms actually overlap as fungi are found in, and contribute to, high soil C:N through a relatively higher average biomass C:N ratio than bacteria (Lauber et al., 2008; Zhang and Elser, 2017).

Carbon Use Efficiency

The lack of microbial CUE response to soil C:N is surprising given the theoretical framework and evidence that predicts microbial CUE to decrease with increasing substrate C:N (Manzoni et al., 2012; Cotrufo et al., 2013). Instead, we observed an increase in microbial CUE with EcM relative abundance, indicating that the free-living microbial community grows more efficiently where EcM are more dominant. The free-living microbes in EcM-dominated soils likely rely more on photosynthate C through mycorrhiza-mediated priming rather than the surrounding SOM (Schimel, 2003; Fransson et al., 2016; Frey, 2019). We therefore suggest that the effects of EcM on the free-living community override the importance of soil N availability for microbial CUE in this system.

Given substantial evidence showing lower soil N content and N mineralization rates in EcM-dominated soils (Phillips et al., 2013; Lin et al., 2017; Cotrufo et al., 2019), we expected to find a negative association between EcM relative abundance and free-living microbial CUE, as microbes are predicted to grow less efficiently with increasing ratios of C to nutrient availability (Manzoni et al., 2012). Moreover, EcM have been shown to compete with free-living saprotrophs for N (i.e., the “Gadgil effect”; Averill et al., 2014), which has been associated with declines in free-living community CUE (Anthony et al., 2020). Instead, in this study, we observed a strong positive correlation between EcM relative abundance and free-living microbial CUE. This positive response could have been driven by considerable EcM exudate secretion to the surrounding microbial community (Phillips and Fahey, 2006; Frey, 2019), outweighing the potential negative effects of nutrient competition between the free-living community and EcM (Phillips and Fahey, 2005; Fransson et al., 2016; Soong et al., 2020). This mechanism is further supported by a study which found that EcM exudate production decreased under elevated soil N conditions, followed by a decrease in microbial growth and biomass (Gorka et al., 2019). These results suggest that soils with greater soil N concentrations, as in our AM-dominated plots, may have less root exudation, which would suppress the biomass production of free-living microbes (Soares and Rousk, 2019). Moreover, it is increasingly recognized that the Gadgil effect is only observed under specific environmental conditions (Fernandez and Kennedy, 2016), such as in systems with highly recalcitrant litter types (e.g., pine litter with high lignin:N and high C:N; Smith and Wan, 2019) and where certain EcM taxa characterized by competitive traits are present (Fernandez et al., 2020). Instead, our results suggest that in the temperate forest soils we studied, EcM may predominantly be involved in the facilitation of free-living microbial growth and efficiency.

While microbial CUE responded positively to EcM relative abundance across horizons, it also decreased with the F:B ratio in the mineral horizon, suggesting divergent effects of different members of the fungal community. This lower CUE in soil with a higher F:B ratio supports other findings that as free-living communities shift to higher F:B ratios, the community becomes less efficient (Silva-Sánchez et al., 2019; Soares and Rousk, 2019). Some studies have found the opposite effect, where CUE increased with increasing fungal dominance or depended on substrate availability; however, these laboratory-based studies added individual simple substrates and did not capture how the soil microbial community interacts with complex native substrates (Lipson et al., 2009; Keiblinger et al., 2010). Importantly, the negative effect of F:B ratio on free-living microbial CUE in the mineral horizon was three times stronger than the positive effect of EcM relative abundance. Consequently, though the effect of EcM fungi on the free-living community CUE may be positive via priming, the higher F:B in soil beneath EcM-associated trees may result in lower overall microbial CUE in mineral soils, as was found in a recent global analysis (Anthony et al., 2020).

A relative increase in soil microbial CUE has been hypothesized as a driver of long-term soil C storage and an important piece of information to include in models projecting future C sequestration potential (Kallenbach et al., 2016). The distribution of AM and EcM trees is projected to change across the continental United States in response to a number of factors including climate change and nitrogen deposition, with an increase in AM dominance in the New England area expected (Jo et al., 2019). Our results show that microbial CUE is strongly related to mycorrhizal association, both positively via EcM priming and negatively due to increasing fungal dominance, with a net negative effect on CUE in mineral soil. Therefore, a potential shift toward AM-dominated forests that would reduce soil EcM relative abundance as well as F:B, could cause conceivable net increases in mineral soil CUE. Increased CUE likely leads to a larger proportion of soil C becoming stored in organo-mineral associations (Cotrufo et al., 2013)—an effect that should be strongest in mineral soil (Craig et al., 2018). Therefore, we support continued efforts to use mycorrhizal associations as an effective and relatively simple proxy for predicting soil carbon dynamics at broad scales (Sulman et al., 2019).

The generalizability of these results needs to be tested at other sites and other times of the year. Our EcM basal area gradient was dominated by Pinaceae in the higher EcM-dominant plots. Consequently, the relationships described here may differ from those in forests dominated by other EcM families such as Fagaceae. Because the microbial functional data were analyzed on soils from only one time point, in the early growing season, it is possible that results could differ at other times of year, such as leaf-fall or the spring thaw. The phenology of mycorrhizal fungi function is very much an open question. We are confident, however, in our fungal community analysis. We also extracted DNA and analyzed ITS data from soils at the same plots in 2018, and we found a very similar EcM relative abundance relationship along the EcM basal area gradient.

CONCLUSION

The trends we observed along our gradient of EcM basal area support the conclusion that fungal community composition is a stronger driver of soil microbial functions than organic matter quality. First, we found that EcM and the saprotrophic free-living community had divergent effects on soil enzyme activities with BG + CB being driven by EcM fungi and AP + LAP and PO being driven by saprotrophic fungi. Second, we found that the decreases in CUE with increasing fungal dominance along the EcM basal area gradient likely outweighed the positive effects of priming by EcM fungi in mineral soil. This latter finding requires further research that disentangles the effects of EcM relative abundance alone versus fungal dominance associated with EcM-associated trees on microbial CUE. Overall, our results indicate

that including mycorrhizal plant functional types in models of the soil carbon cycle is warranted and future modeling efforts should focus on integrating the fungal community.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are be found in the ESS_Drive repository: <https://data.ess-dive.lbl.gov/view/doi:10.15485/1659374>.

AUTHOR CONTRIBUTIONS

AF led field sample collection and processing, and data analysis and writing. AL and EW contributed to interpreting results and writing. EW also led bioinformatics for the ITS data. KG contributed to CUE methods optimization, and CUE data production and analysis. CH conceived of the experimental design and contributed to data analysis and writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2020.569945/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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