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ARTICLE

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Forest structural diversity is linked to soil microbial diversity

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Abstract

Efforts to catalog global biodiversity have often focused on aboveground taxonomic diversity, with limited consideration of belowground communities. However, diversity aboveground may influence the diversity of belowground communities and vice versa. In addition to taxonomic diversity, the structural diversity of plant communities may be related to the diversity of soil bacterial and fungal communities, which drive important ecosystem processes but are difficult to characterize across broad spatial scales. In forests, canopy structural diversity may influence soil microorganisms through its effects on ecosystem productivity and root architecture, and via associations between canopy structure, stand age, and species richness. Given that structural diversity is one of the few types of diversity that can be readily measured remotely (e.g., using light detection and ranging—LiDAR), establishing links between structural and microbial diversity could facilitate the detection of belowground biodiversity hotspots. We investigated the potential for using remotely sensed information about forest structural diversity as a predictor of soil microbial community richness and composition. We calculated LiDAR-derived metrics of structural diversity as well as a suite of stand and soil properties from 38 forested plots across the central hardwoods region of Indiana, USA, to test whether forest canopy structure is linked with the community richness and diversity of four key soil microbial groups: bacteria, fungi, arbuscular mycorrhizal (AM) fungi, and ectomycorrhizal (EM) fungi. We found that the density of canopy vegetation is positively associated with the taxonomic richness (alpha diversity) of EM fungi, independent of changes in plant taxonomic richness. Further, structural diversity metrics were significantly correlated with the overall community composition of bacteria, EM, and total fungal communities. However, soil properties were the strongest predictors of variation in the taxonomic richness and community composition of microbial communities in comparison with structural diversity and tree species diversity. As remote

sensing tools and algorithms are rapidly advancing, these results may have important implications for the use of remote sensing of vegetation structural diversity for management and restoration practices aimed at preserving belowground biodiversity.

KEYWORDS
canopy complexity, forest structure, LiDAR, microbial biogeography, mycorrhizal fungi, remote sensing, soil bacteria, soil biodiversity, soil fungi

INTRODUCTION

Decreases in biodiversity are occurring in nearly all regions on Earth, with critical consequences for how ecosystems function (Díaz et al., 2019; Hooper et al., 2012; Isbell et al., 2022; Loreau et al., 2001). Most research on biodiversity loss has focused on vascular plants (Tydecks et al., 2018), partly due to the ease with which plant species diversity can be measured. In contrast, far less is known about the diversity of belowground communities (e.g., soil microbes), despite their importance to ecosystem functions such as decomposition, nutrient retention, and feedbacks to the climate system. One reason for our limited understanding of microbial community diversity is the greater amount of time and resources needed to characterize microbial communities compared with other groups of organisms (Kirk et al., 2004; Maron et al., 2011), which limits our ability to make inferences.

Theory suggests that diversity at one trophic level should be positively associated with diversity at another trophic level. However, it is less clear whether this pattern applies to plants and their associated soil microbial communities (Fei et al., 2022). Plants are likely to influence soil microbial communities through resource allocation to belowground structures, litter production, and through plant–fungal interactions (i.e., mycorrhizal associations). Because of these close relationships between plants and soil microbes, plant communities may be useful surrogates for estimating soil microbial diversity (Westgate et al., 2017). However, much of the research linking above- and belowground diversity in forests has been based on tree species richness (e.g., Li et al., 2020), but previous work has found equivocal patterns in the direction and strength of the relationships between tree species richness and microbial diversity, including that of mycorrhizal fungi (Fei et al., 2022; Wagg et al., 2015).

The three-dimensional (3D) volume and arrangement of vegetation within the ecosystem (structural diversity) is an overlooked aspect of ecological diversity (LaRue et al., 2023) that may also be linked to soil microbial diversity. Structural diversity, particularly in forests, can be estimated with remote sensing (LaRue et al., 2020; Lim et al., 2003; Mura et al., 2015) and provides an opportunity to capture multiple aspects of biodiversity (D'Urban Jackson et al., 2020; Valbuena et al., 2020). Structural diversity represents functional variation in plant size that creates habitat and supports ecosystem functions that are linked to the biodiversity of soil organisms (Taboada et al., 2010). Of particular interest are potential linkages between the structural diversity of vegetation and the diversity of soil microbial communities, two groups of organisms known to exert strong controls on ecosystem productivity and biogeochemical cycling (Wagg et al., 2011, 2019; Zak et al., 2003). Yet, it is still unclear whether the structural diversity of plant communities is related to the diversity of soil bacteria and fungi, and whether these remotely sensed metrics of aboveground structural diversity may be used to predict soil microbial diversity and belowground ecosystem processes.

There are several ways in which forest structural diversity may directly or indirectly affect soil microbial diversity (Figure 1). First, aboveground structural diversity should be positively linked to belowground structural diversity. More structural complexity belowground may result from a wider variety of root morphologies, including branching architecture and rooting depths, that provide distinct microbial habitats and thus may support a more diverse microbial community (McCormack et al., 2015). Second, structural diversity is known to be positively associated with higher light capture and complementary resource use by trees, which corresponds with higher forest productivity (Gough et al., 2019; Ishii et al., 2004). Structural diversity may therefore enhance carbon fixation and, subsequently, root carbon exudation and carbon allocation to mycorrhizal fungi that fuel both the soil decomposer and mycorrhizal fungal communities (Anthony et al., 2022). Third, structural diversity may be associated with other characteristics of the forest, including stand age structure and tree species richness, that influence soil microbial diversity (Parker & Russ, 2004; Wales et al., 2020). For example, both above- and belowground structural diversity change as trees age (Matsuo et al., 2021), and the communities of root-associated microbial taxa shift with tree age and nutrient demand

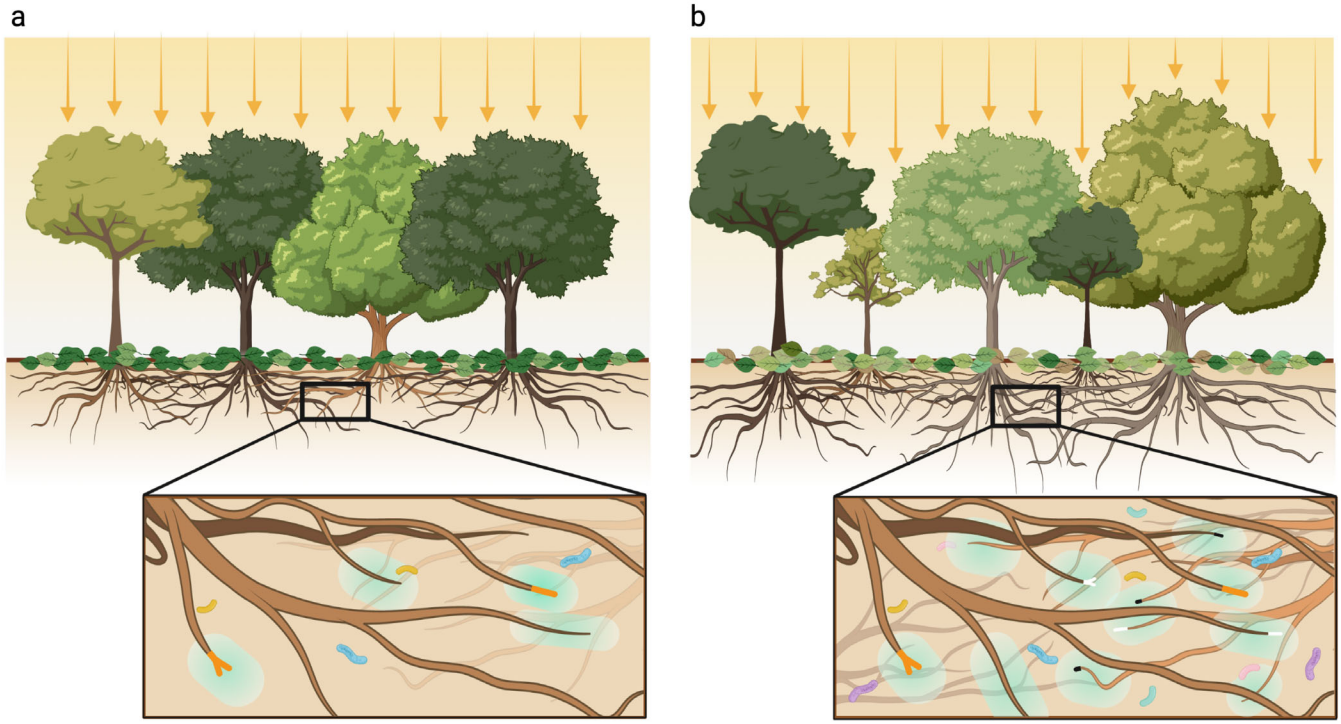

FIGURE 1 Forest structural diversity may influence soil microbial diversity through several pathways. First, a less structurally diverse forest (a) may support lower soil microbial diversity than a structurally complex forest (b) due to less effective light capture (yellow arrows), leading to lower net primary productivity and less belowground carbon allocation to microbial communities (green shaded regions in inset figures indicate root carbon exudation). Lower aboveground structural complexity may also be associated with less complex rooting architecture, providing fewer niches for soil microorganisms. Indirectly, changes in structural complexity at the stand level may be associated with stand age and evenness of tree age classes, or with tree species richness, both of which likely influence soil microbial community composition and richness.

(Gange et al., 1993; Johnson et al., 2005). Younger and more even-aged stands are therefore likely to have a lower degree of structural complexity both above- and belowground, and hence a less diverse soil microbial community, compared with older stands with multiple cohorts of trees. Further, forests with a greater number of tree species are more likely to have more complex canopies and root morphologies, provide more diverse organic matter inputs to the soil microbial community, and support a larger variety of mycorrhizal associations (Singavarapu et al., 2022; Steinauer et al., 2016; Tedersoo & Bahram, 2019). Through these pathways, whether directly or mediated by changes in stand age and composition, changes in the structural diversity of forest canopies may have cascading impacts on the composition of soil bacterial and fungal communities.

Microbial guilds, including mycorrhizal fungal guilds, may respond to changing forest structural diversity via distinct mechanisms. For example, canopy complexity may influence the community of decomposers through corresponding changes in tree productivity and biomass that ultimately influence the rate of organic matter inputs to the forest floor (Nguyen et al., 2016). Because canopy complexity is associated with forest productivity (Gough et al., 2019), more structurally diverse forests may support larger pools of coarse woody debris and faster root turnover due to higher rates of tree growth, supplying decomposers with more substrate than forests with less complex canopies. Patterns in the community structure of mycorrhizal fungi, however, are more likely to respond to canopy complexity via changes in the trait diversity of the tree species in a stand. Because different mycorrhizal functional guilds tend to associate with certain tree species and mycorrhizal communities tend to differ with tree age class (Aučina et al., 2011; Brundrett, 2004; Ferlian et al., 2021; Johnson et al., 2005; Nguyen et al., 2016; van der Linde et al., 2018), the most important effects of canopy structure are likely due to corresponding changes in tree species richness, forest age structure, or in traits that influence the formation of mycorrhizal associations, including root morphology. Further, more productive host trees tend to supply mycorrhizal fungi with larger quantities of carbon, so faster growing, structurally complex forests may also harbor distinct communities of mycorrhizal fungi with higher carbon demand compared with slower growing stands (Anthony et al., 2022). Therefore, while bacterial and fungal decomposers may be influenced most by the variety of substrates available

in highly structurally diverse forests, mycorrhizal fungi may be linked with variation in tree traits, species richness, and forest stand age (Anthony et al., 2022; Birch et al., 2021; Comas et al., 2014).

To identify potential linkages between above- and belowground diversity, we tested possible correlations between forest structural and soil microbial diversity in 38 plots across the central hardwood region in Indiana, USA. We expected that structural diversity would be a significant predictor of soil bacterial and fungal (including mycorrhizal fungal) richness (alpha diversity) and community composition. We expected these relationships to be stronger for the total fungal community and bacterial communities relative to the mycorrhizal fungal communities due to the direct pathways by which canopy complexity fuels the production of decomposition substrates. We also predicted that connections between plant structural diversity and soil microbial diversity would be equally strong or stronger than relationships between plant richness and microbial diversity. We also examined the relative predictive ability of tree species richness (Wu et al., 2019), stand age and productivity (Högberg et al., 2007; Wagg et al., 2011), climate (Nottingham et al., 2018; Pold & DeAngelis, 2013), and soil properties to explain variation in soil microbial richness and community composition. In particular, we included several soil factors with known effects on microbial community composition, including pH (Davison et al., 2021; Rousk et al., 2009), carbon-to-nitrogen ratio (Midgley & Phillips, 2016; Soares & Rousk, 2019), and mineral composition (represented by oxalate-extractable iron content; Carson et al., 2009; Whitman et al., 2018).

Remote sensing technologies that can resolve 3D structural diversity (e.g., light detection and ranging—LiDAR) are becoming readily available from landscape to global scales (Zeng et al., 2022). Identifying linkages between above- and belowground diversity will provide the potential to map indicators of belowground diversity across large spatial scales, which could become an important tool for managing ecosystem services and soil biodiversity that might otherwise be difficult to monitor without time-consuming genomic and chemical analyses of soils (Bakker et al., 2019).

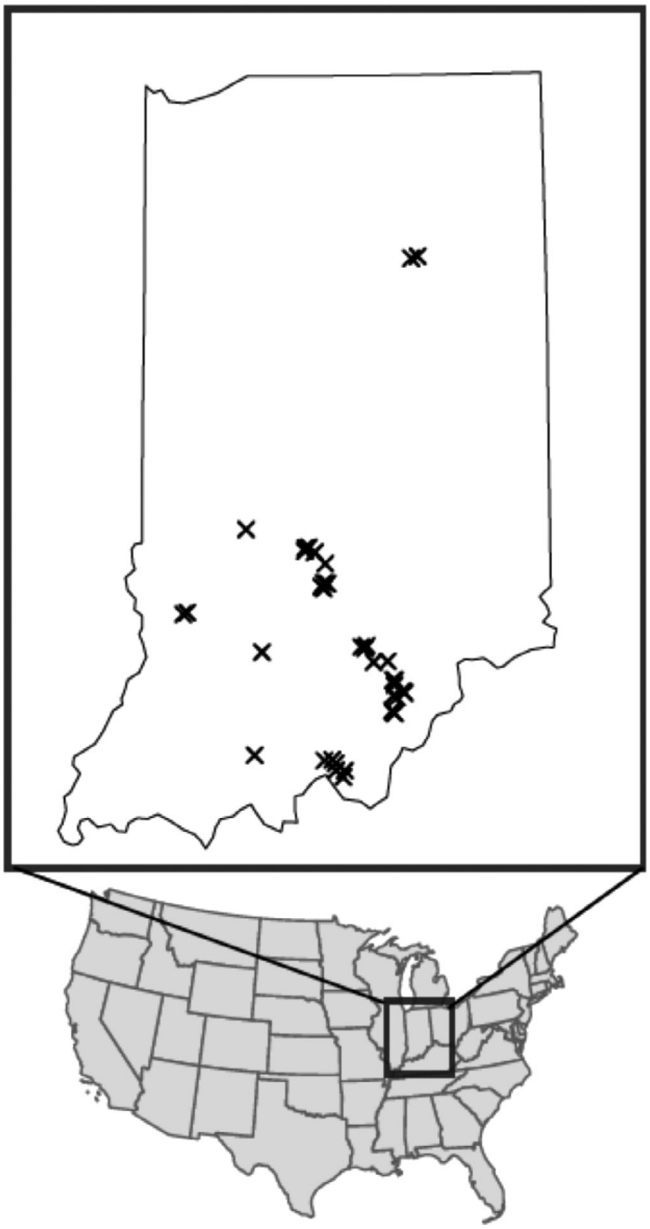

FIGURE 2 Location of study sites within the central hardwood region of Indiana, USA ($N_{plots} = 38$).

MATERIALS AND METHODS

Forest structural diversity and stand properties

We obtained inventory data on 38 forest plots from the Indiana Continuous Forest Inventory (CFI) (Gallion, 2018) in the central hardwoods region of Indiana, USA (Figure 2). The dominant tree species in this area are deciduous hardwoods, including red maple (*Acer rubrum*), sugar maple (*A. saccharum*), American beech (*Fagus grandifolia*), white oak (*Quercus alba*), and yellow poplar (*Liriodendron tulipifera*). Coniferous tree species, including red pine (*Pinus resinosa*), Virginia pine (*P. virginiana*), eastern white pine (*P. strobus*), and eastern red cedar (*Juniperus virginiana*), constituted roughly 7% of the stems in our data set and were found in 9 of the 38 study plots. Individual trees in each CFI plot were identified to species and diameter at breast height is measured every five years. We estimated tree species richness and productivity with individual stem-level data from the growing season of 2020 (May–October). Tree species

richness was calculated as the number of unique tree species found within each 7.3-m-radius circular plot. The change in basal area of trees over the five-year interval was used as a predictor of plot productivity, which was calculated as the annual increase in basal area from 2015 to 2020. Stand age was determined by tree ring analysis of a focal tree at the time of plot establishment. If the stand was too young and no trees were suitable for coring, stand age was determined with information from forest managers and land owners. Finally, the type of mycorrhizal association for each tree host was classified following Jo et al. (2018) and used to calculate ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) host richness and abundance. The dominance of AM trees was calculated by dividing the AM tree basal area by the sum of AM and EM tree basal area (Jo et al., 2018).

To quantify the aboveground structural diversity of forests, we obtained discrete return LiDAR from the 2017 to 2019 USDA 3DEP survey (USGS, 2020). Details about the collection and specifications of the 3DEP LiDAR can be found on the USDA 3DEP website (https://www.usgs.gov/3d-elevation-program). A 30-m-radius buffer area was clipped around the plot centroid. Large groups of atmospheric and ground outliers were filtered by removing points above and below six SDs of the mean height and then manually checked to ensure that outliers were actually removed. All LiDAR processing was conducted in the lidR (version 3.1.2) R package (Roussel et al., 2020; Roussel & Auty, 2022). The buffer area was then corrected for elevation using a Delaunay triangulation before being clipped to a 2.22-m-radius circular plot. Three structural diversity metrics were calculated from each plot area that represent the volume and arrangement of structural diversity in forests (LaRue et al., 2020). These metrics were chosen based on stability across different LiDAR point densities (i.e., 2–8 points per m^2) (LaRue et al., 2022). Points below 0.5 m were filtered from the point cloud to exclude ground points and the following metrics were calculated: the SD of the height of points, vegetation area index (VAI), and vertical complexity index (VCI). VAI describes the density of vegetation within forest canopies and was calculated with the leaf area density (LAD) function from the lidR package (Roussel et al., 2020). The standard deviation of vegetation heights (VertSD) and VCI describe the vertical heterogeneity of vegetation throughout the vertical canopy profile. VertSD was calculated from the cloud_metrics function and VCI from the VCI function in the lidR package.

Microbial diversity and soil chemistry

Soil samples were collected from each study plot in accordance with protocol from the Indiana CFI program (Gallion, 2018). Two soil cores (~200 cm^3) were collected on the perimeter of each plot in 2020 during the growing season; one core was collected each from the east and west sides of the plot. The cores were subdivided into two depths (0–5 cm and 5–10 cm) and homogenized within depths at the time of collection. Samples were air-dried and passed through a 2-mm sieve prior to chemical and microbial analyses. The mass percentage of carbon and nitrogen in each sample was determined using an elemental combustion system (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA, USA). Oxalate-extractable iron content, a proxy for mineral soil reactivity, was determined using a 200 mg subsample of soil from each plot and each depth, and was extracted with a 0.2 M ammonium oxalate solution. Oxalate-extractable iron concentration was determined on a mass percent basis using atomic absorption spectrometry (PerkinElmer Instruments, Waltham, MA, USA).

DNA was extracted from ~250 mg of homogenized soil from 0–5 cm and 5–10 cm core depths of each plot using the Qiagen DNeasy Soil Extraction kit (Qiagen, Germantown, MD, USA). DNA was quantified with the Qubit high sensitivity kit (Qubit Fluorometer, Life Technologies, Carlsbad, CA, USA) and diluted to ~10 ng/μL in sterile water. We amplified fungi using barcoded 5.8-Fun and ITS4-Fun primers targeting the internal transcribed spacer 2 (ITS2) region (Taylor et al., 2016), and bacteria via barcoded S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 primers of the 16S region (Klindworth et al., 2013). Each polymerase chain reaction (PCR) contained 5 μL of ~1–10 ng/μL DNA template, 21.5 μL of Platinum PCR SuperMix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1.25 μL of each primer (10 μM), 1.25 μL of 20 mg/mL bovine serum albumin (BSA), and 0.44 μL of 25 mM $MgCl_2$. For the ITS2 primers, the reactions included an initial denaturing step at 96°C for 2 min, followed by 24 cycles of 94°C for 30 s, 51°C for 40 s, and 72°C for 2 min, with a final extension at 72°C for 10 min. For the 16S primers, reactions started with an initial denaturing step at 95°C for 5 min, followed by 25 cycles of 95°C for 40 s, 55°C for 2 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. To accurately capture the AM fungal community, we amplified AM fungal DNA separately. Due to limited AM fungal DNA, we first performed a nested PCR reaction. The first reaction amplified an ~800 bp region of AM fungal and plant DNA in the 18S region using the NS1–NS4 primers (White et al., 1990), the preferred marker gene for AM fungi (Lekberg et al., 2018). The nested reaction amplified an ~400 bp region of 18S AM fungal DNA with barcoded Illumina TruSeq version 3 indices (Illumina, San Diego, CA, USA) linked to the

NS31-AML2 primers (Morgan & Egerton-Warburton, 2017). Each reaction contained: 21.5 μL of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 μL of each primer (10 μM), 0.5 μL of BSA (20 mg/mL), and 2 μL (~20 ng) of DNA. The first reaction ran at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 40°C for 1 min, and 72°C for 1 min and the nested reaction at 94°C for 5 min, followed by 40 cycles of 94°C for 45 s, 63.1°C for 1 min, and 72°C for 1.5 min. In all cases, triplicate reactions were combined, cleaned with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), and quantitated fluorometrically (Qubit Fluorometer, Life Technologies, Carlsbad, CA, USA). Samples were pooled into equal amounts and run on an Illumina MiSeq version 3 sequencer in a 2×300 bp run at the University of Tennessee Center for Environmental Biotechnology core.

All sequences were processed in the DADA2 pipeline in R (Callahan et al., 2016). First, primers were trimmed from all sequences and sequence error rates were calculated. Sequences were then merged into unique amplicon sequence variants (ASVs). Finally, chimeras were removed using a denovo chimera checker. ASVs were not clustered prior to assigning taxonomy, thus every sequence variant was included in downstream analysis (Glassman & Martiny, 2018). For general fungal communities (ITS) and bacterial communities (16S, short-term study only), we used the default DADA2 classifier (Wang et al., 2007) to assign taxonomy based on reference sequences from the UNITE database version 9.0 (Nilsson et al., 2019) for ITS sequences and the SILVA database R138.1 (Quast et al., 2013) for *16S* sequences. We identified AM fungi by BLASTing representative sequences from ASVs against the MaarjAM database (Opik et al., 2010), only retaining reads with at least a 97% match for a known AM fungal virtual taxonomic unit.

Universal ITS barcode primers are known to discriminate against early-diverging fungal lineages, like AM fungi (Stockinger et al., 2010), so we do not make direct comparisons between ITS-derived and small-subunit-derived data for AM fungi. EM fungi were defined via the FungalTraits database (Põlme et al., 2020). All ASV read data were relativized, rather than rarified (McMurdie & Holmes, 2014), prior to downstream alpha diversity and community composition analyses. Sequences are deposited in the NCBI Sequence Read Archive (Edwards & Kivlin, 2023). Minimum, maximum, average, and SE read number for each group at each processing step are provided in Appendix S1: Table S1.

We calculated taxonomic richness (alpha diversity) and community composition of bacteria, total fungi, and EM and AM fungal groups. We calculated alpha diversity with the inverse Simpson's index using the diversity function in the vegan package in R (Oksanen et al., 2022); although we tested several diversity metrics for this analysis, most metrics yielded qualitatively similar results, so we focused on inverse Simpson's index based on recommendations for mycorrhizal fungi (Morris et al., 2014) and for simplicity of interpretation (i.e., larger values of Simpson's index indicate greater alpha diversity). We calculated community composition using the quantitative Jaccard index with the vegdist function in the vegan package (Oksanen et al., 2022).

Statistical analyses

To test for relationships between variables related to aboveground vegetation and soil conditions and alpha diversity of the soil microbes, we constructed a suite of eight general linear regression models to test for significant relationships between microbial alpha diversity (species richness) and vegetation and soil conditions; separate models for alpha diversity were developed for each microbial guild (bacteria, all fungi, EM fungi, and AM fungi) at a given depth (0–5 cm and 5–10 cm). We calculated partial R^2 values using the sensemakr package in R (Cinelli et al., 2021) and scaled model coefficients to allow for comparison of the strengths of model predictors (Gelman, 2008). Before developing the general linear regression models, we removed predictor variables that had a correlation coefficient greater than 0.70 (Appendix S1: Figure S1; Tabachnick & Fidell, 2013). Variable selection was based on relevance to our hypothesized drivers and designed to optimize the amount of information gained by keeping specific predictors in the model (Gregorich et al., 2021). For example, AM and EM tree species richness were highly correlated with total tree species richness and AM dominance, so we removed AM and EM tree richness because total tree species richness allowed us to preserve more information about plant community richness, and because AM dominance reflects the relative importance of both AM and EM tree species in each plot based on basal area. However, we also assessed correlations between microbial diversity and AM and EM host tree richness to ensure that patterns identified in our analyses were not due to underlying relationships between stand properties and host tree richness (Appendix S1: Figure S1). Finally, we evaluated our models with and without the random effect of county to account for spatial patterns in the locations of sampling plots. The effect of county was negligible in all models (Appendix S1: Table S2), and therefore, we present the more parsimonious linear models without a random effect of county.

Next, we tested the significance and relative strength of structural diversity and other environmental variable categories for their ability to explain the variation in

microbial community composition (i.e., community similarity among sites) at both 0–5 cm and 5–10 cm soil depths. We conducted a distance-based redundancy analysis (dbRDA) using the dbRDA function using the vegan package in R (Oksanen et al., 2022) to assess patterns in the composition and structure of the total fungal community, the bacterial community, the AM fungi, and EM fungi. Factors contributing to the variation in community composition were partitioned among four categories (Table 1) and we ran models with variables grouped into these categories and also separately to assess individual variable significance. For both variable groups and individual variables, we ran full dbRDA models with all terms, then ran subsequent reduced models with only the terms with $p < 0.05$ from the full model. Variation in community composition due to spatial autocorrelation among plots was detrended prior to analysis. The explanatory power of each category indicated as significant by the dbRDA model was assessed using the varpart function in the vegan package. We used variance partitioning analysis to assess the relative importance of different drivers of site-to-site variation in overall community composition and structure, following recommended procedure for analyses of community composition (Legendre, 2008). Highly correlated variables within each category were removed before analysis (see Appendix S1: Figure S1: AM and EM tree richness, soil %N, soil %C). Data and R code for all analyses are available online (Lang, 2023).

TABLE 1 Soil-, plant-, and stand-level variables predicted to be linked to microbial composition and diversity in forest soils.

Category	Variable	Unit
Structural diversity	Standard deviation of vegetation height (VertSD)	m
	Vegetation area index (VAI)	m^2/m^3
	Vertical complexity index (VCI)	Unitless
Tree diversity	Tree species richness	Species no.
	AM dominance	Proportion
	AM tree richness	Species no.
	EM tree richness	Species no.
Stand productivity and age	Basal area increment (BAI)	m^2/year
	Stand age	Years
Soil properties	Soil pH	Unitless
	C:N ratio	Unitless
	Oxalate-extractable iron (Fe_{Ox})	Percent

Note: The effect of each variable was assessed individually in models of microbial species richness and in groups for models of microbial community composition. Structural diversity variables were calculated from LiDAR data, tree diversity, and stand productivity and age data were gathered from the Indiana Continuous Forest Inventory (CFI) project, and soil properties were measured from samples collected in each CFI plot in the growing season of 2020.

Abbreviations: AM, arbuscular mycorrhizal; EM, ectomycorrhizal; LiDAR, light detection and ranging.

RESULTS

Stand and soil characteristics

Across the 38 plots, tree species richness ranged from 1 to 6 species, and both AM and EM tree mycorrhizal types were well represented; AM tree basal area percentage ranged from 0 to 100, with a mean value of 52.2%. Stands ranged in age from 15 to 126 years at the time of soil sampling. Stand age was negatively associated with AM tree dominance and soil pH, and positively associated with EM tree species richness (Appendix S1: Figure S1).

Mean soil C content was 2.95% and mean N content was 0.19%. C:N ranged from 10 to 28.5. Soil pH ranged from 3.6 to 6.4, and the mass percentage of oxalate-extractable iron ranged from 0.1% to 0.86%. Soil pH was positively associated with iron content, %C and % N, and C:N, as well as with AM tree species dominance (Appendix S1: Figure S1).

Microbial community composition

In the fungal communities, Ascomycota were the most abundant phylum, averaging 41.9% of fungal communities across all samples. Basidiomycota were the second most abundant fungal phylum with 34.6% relative abundance on average, followed by Murcoromycota (13.9%), Mortierellomycota (6.8%), and Chytridiomycota (1%; Appendix S1: Figure S2). The most abundant fungal genera on average were Umbelopsis (10.2%), Russula (7%), Mortierella (4.2%), Geminibasidium (2.7%), and Lactifluus (2.4%). Ectomycorrhizal communities were derived from general fungal communities; thus, their relative abundance may be less informative, but the most abundant genera designated as EM-associated were as follows: Russula, Cenococcum, Tomentella, Inocybe, and Inosperma. Among the five most abundant AM fungal taxa, four were Glomeraceae *Glomus* sp. (VTX 00084, 00392, 00214, and 00199) cumulatively comprising an average of 18.5% of the AM fungal community. The second most abundant AM fungal taxon was Acaulosporaceae *Acaulospora* sp. VTX 00026 with an average of 4.6% of the AMF community.

In bacterial communities, Proteobacteria were the phylum with the greatest relative abundance, averaging 25.2% across all samples. Acidobacteria were the second most abundant with an 18.2% average relative abundance, followed by Actinobacteriota (14.8%), Verrucomicrobiota (11%), and Chloroflexi (10.9%; Appendix S1: Figure S2). The most abundant bacterial genera were Candidatus Udaeobacter (6.2%), Xanthobacteraceae genera (5.2%), Elsterales genera (4.9%), Gemmataceae genera (4.4%), and Acidobacteriales genera (3.9%).

Linking forest structural diversity with microbial richness and diversity

Forest structural diversity was associated with microbial richness (alpha diversity) and community composition to varying degrees. First, VAI was positively associated with EM fungal richness in the upper surface soils (Table 2, Figure 3). No other metrics of canopy structural complexity (VCI, VertSD) were associated with the alpha diversity of the soil microbial communities.

Second, individual metrics of forest structural diversity were associated with several components of microbial community composition. VAI and VertSD predicted variation in EM fungal communities at both soil depths and in the total fungal community at 5–10 cm depth (Appendix S1: Table S3). Collectively, structural diversity variables significantly predicted variation in the composition of the bacterial and EM fungal communities in both soil depths and the total fungal community in the 0–5 cm depth (Table 3).

TABLE 2 Effects of plant community, productivity, canopy structure, and soil properties on the alpha diversity of soil microbial communities calculated with the inverse Simpson's index.

	0–5 cm				5–10 cm			
Variable	**Bacteria**	**Total fungi**	**AM fungi**	**EM fungi**	**Bacteria**	**Total fungi**	**AM fungi**	**EM fungi**
Total tree richness	...	...	...	...	...	...	...	...
AM dominance	...	...	...	...	...	...	...	...
Stand age	...	...	...	$\beta = 0.58$ $R^2 = 0.21$ $t_{25} = 2.55$ $p = 0.017$	...	...	...	...
BAI	...	...	...	...	...	$\beta = 0.41$ $R^2 = 0.29$ $t_{25} = 3.18$ $p = 0.004$	...	...
VertSD	...	...	...	...	...	...	...	...
VAI	...	...	...	$\beta = 0.41$ $R^2 = 0.19$ $t_{25} = 2.43$ $p = 0.023$	...	...	...	...
VCI	...	...	...	...	...	...	...	...
C:N	...	...	...	...	...	...	...	...
pH	$\beta = 0.62$ $R^2 = 0.37$ $t_{27} = 4.02$ $p < 0.001$	...	$\beta = 0.43$ $R^2 = 0.18$ $t_{24} = 2.32$ $p = 0.029$	...	$\beta = 0.63$ $R^2 = 0.25$ $t_{25} = 2.90$ $p = 0.008$	$\beta = 0.32$ $R^2 = 0.16$ $t_{25} = 2.15$ $p = 0.042$	$\beta = 0.61$ $R^2 = 0.19$ $t_{22} = 2.28$ $p = 0.032$	...
Fe_{Ox} (percent)	...	...	...	...	...	...	...	...
N_{plots}	38	37	35	36	36	36	33	36

Note: Linear coefficients (β) indicate the strength and direction of the effects of model parameters and are standardized by dividing by two SDs to allow for comparison of the strength of drivers within each model. Partial R^2 and p values are reported only for trends significant at $\alpha = 0.05$. Variable descriptions and abbreviations are defined in Table 1.

Abbreviations: AM, arbuscular mycorrhizal; EM, ectomycorrhizal.

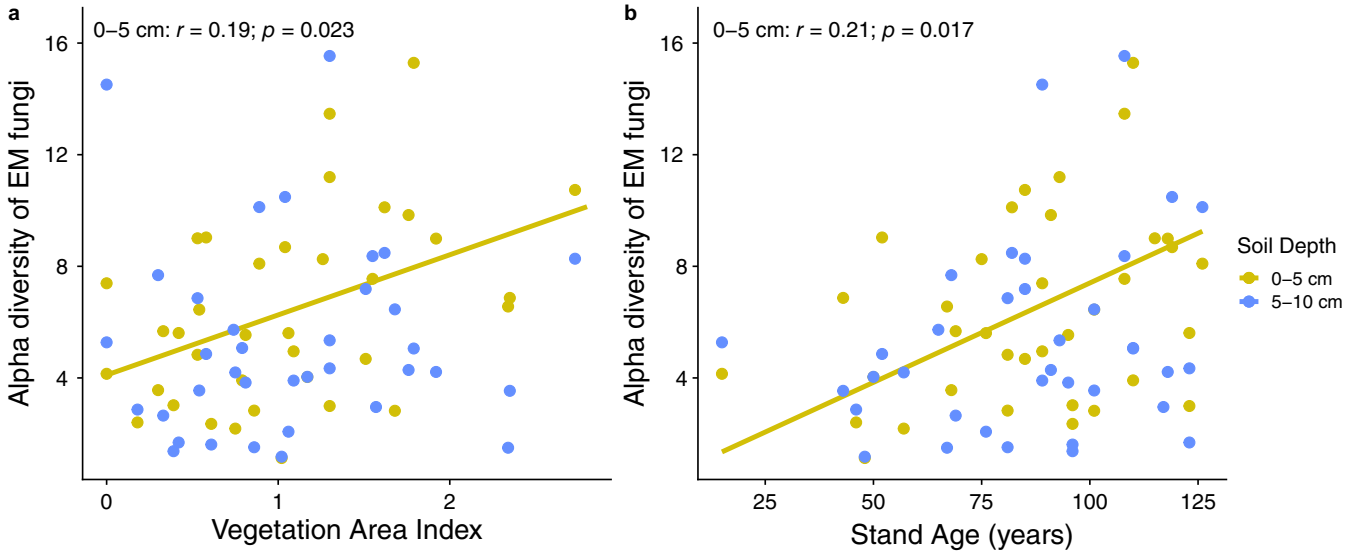

FIGURE 3 Relationship between vegetation area index (VAI) and forest stand age and alpha diversity of ectomycorrhizal (EM) fungi, calculated with the inverse Simpson's index. Points represent measured values of microbial richness at 0–5 cm and 5–10 cm soil depths and VAI and stand age of plots in forests across Indiana; solid line represents the marginal effects of each predictor independent of the effects of other structural diversity variables, and is plotted only for soil depths where the relationship was statistically significant ($\alpha = 0.05$; Table 2).

TABLE 3 Significant predictors of microbial community composition explained by structural diversity and environmental variables in a distance-based redundancy analysis (dbRDA).

		0–5 cm				5–10 cm			
Variable group	**df**	**Bacteria**	**Total fungi**	**AM fungi**	**EM fungi**	**Bacteria**	**Total fungi**	**AM fungi**	**EM fungi**
Structural diversity	2	χ^2: 8.84	χ^2: 11.7	...	χ^2: 39.8	χ^2: 8.42	...	...	χ^2: 71.6
		$p = 0.031$	$p = 0.008$		$p < 0.001$	$p = 0.038$			$p < 0.001$
Tree diversity	3	χ^2: 9.92	χ^2: 14.8	...	χ^2: 67.5	χ^2: 13.1	χ^2: 15.7	χ^2: 26.0	χ^2: 50.7
		$p = 0.007$	$p < 0.001$		$p < 0.001$	$p = 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Stand productivity and age	2	...	...	...	χ^2: 18.8	...	...	χ^2: 15.9	χ^2: 84.5
					$p < 0.001$			$p < 0.001$	$p < 0.001$
Soil properties	3	χ^2: 106	χ^2: 59.1	χ^2: 194	χ^2: 547	χ^2: 87	χ^2: 129	χ^2: 275	χ^2: 280
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Total df		35	36	37	35	33	35	35	35

Note: χ^2 and p values are reported only for a predictor category that explains significant variation in the community composition at $\alpha = 0.05$. The specific predictors within each variable group are described in Table 1. Spatial autocorrelation was accounted for by conditioning all dbRDA models on geographical coordinates and distance-based Moran's eigenvectors.
Abbreviations: AM, arbuscular mycorrhizal; EM, ectomycorrhizal.

Linking soil and stand properties with microbial richness and diversity

Soil properties were associated with both the richness and composition of the bacterial, AM fungal, and total fungal communities, but did not have a consistent effect on the EM fungal community. Soil pH was positively associated with the richness of AM fungi, bacteria, and the total fungal community in both the 0–5 cm and the 5–10 cm soil depths (Table 2; Appendix S1: Figure S3). Similarly, soil properties were the strongest set of predictors of microbial community composition relative to aboveground diversity predictors, with the exception of EM fungal composition (Figure 4).

Stand age and productivity primarily influenced the fungal, rather than bacterial, community richness and composition, particularly for mycorrhizal guilds. The total fungal community richness at 5–10 cm depth was

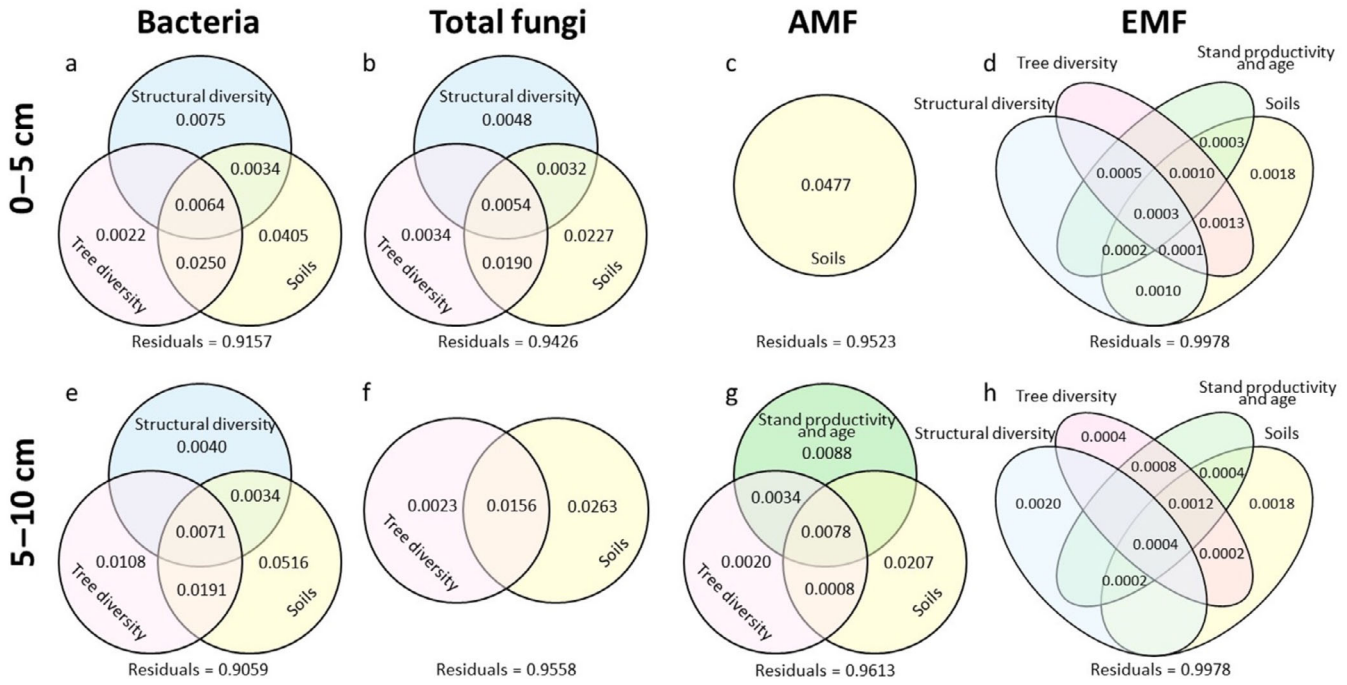

FIGURE 4 Variance explained by each category of predictors that were indicated significant ($p < 0.05$) in the distance-based redundancy analysis model at 0–5 cm (a–d) and 5–10 cm (e–h) soil depths for bacterial community (a, e), total fungal community (b, f), arbuscular mycorrhizal fungi (AMF) community (c, g), and ectomycorrhizal fungi (EMF) community (d, h). See Table 3 for model χ^2 and p values for each significant predictor category.

positively associated with basal area increment, and older stands had greater EM fungal richness at 0–5 cm depth (Figure 3, Table 2). Stand age and productivity significantly influenced the community composition of both AM and EM fungi (Table 3, Figure 4), although this effect was apparent for AM fungi only in the deeper soil (5–10 cm).

DISCUSSION

We expected that structurally diverse forests would provide a greater variety of habitats and resources for soil organisms, which would promote higher taxonomic diversity of soil bacteria and fungi (Figure 1). In partial support of this hypothesis, we found that canopy density was positively related to the alpha diversity of one microbial guild (EM fungi) but not the other three groups we examined here. Further, structural diversity, like tree richness, was a significant predictor of microbial community composition for bacteria, the EM fungal community, and the total fungal community, but neither structural nor taxonomic diversity explained as much variance in microbial community composition as did soil characteristics. Overall, of the four groups of microbes we tested, forest canopy structure had the most consistent effect on the richness and composition of the EM fungi. Collectively, our results suggest that measures of structural diversity, which can be generated remotely, frequently, and across full landscapes, may improve the estimates of the diversity and composition of soil microbes in conjunction with soil chemistry data.

The patterns identified here suggest that the communities of EM fungi are in part shaped by the aboveground complexity of vegetation in forest ecosystems. However, the mechanisms behind this relationship remain unclear, particularly given that these relationships may be mediated by stand age or tree species composition. Although canopy density has been associated with more productive stands (Gough et al., 2019; Hardiman et al., 2011), which may influence microbial community composition (Anthony et al., 2022), vegetation density (VAI) was not strongly correlated with stand productivity in our study system. However, VAI was positively correlated with stand age, and stand age was positively correlated with EM alpha diversity in the surface soil (Table 2). This suggests that older stands have denser, more vertically heterogeneous canopies (Franklin & Van Pelt, 2004; LaRue et al., 2023) and may indicate an uneven age structure of trees that supports a richer community of root-associated EM fungi. We may have only seen patterns between structural metrics and EM fungi, rather than other groups of microbes, because the EM fungi are the only taxa we examined that are closely linked with

only the tree species in each plot: free-living soil microbial taxa should not be as closely linked with tree species characteristics, and AM fungi can associate with both trees and herbaceous plants, likely dampening the influence of the tree canopy on AM fungal richness. Further, the composition of EM fungal communities changes as their host trees age (Birch et al., 2021; Reverchon et al., 2012); uneven-aged stands contain a larger variety of host ages and therefore more opportunities for the establishment of different EM taxa. Notably, although older stands did support greater species richness of EM-associated trees, EM fungal richness was not associated with EM tree species richness, meaning that this pattern is unlikely to be due to concomitant changes in host species richness with stand age (Appendix S1: Figure S4). Further, EM fungi were the only group wherein microbial alpha diversity was not significantly related to the soil pH, further highlighting that while other microbial taxa are strongly influenced by soil conditions, EM fungal richness may be estimated with tree- and stand-level forest composition data. Together, these results suggest that structurally dense and older forest stands may be associated with higher EM taxonomic richness in central hardwood forests.

Soil properties, particularly pH, were the best predictors of alpha diversity and community composition of bacteria, AM fungi, and the total fungal community. These strong relationships may be explained by the physiological tolerances of these taxa; bacteria in particular have been shown to be more sensitive than fungi to soil pH (de Vries et al., 2012; Mitchell et al., 2010; Porter et al., 1987; Rousk et al., 2009). Further, soil conditions like the ratio of carbon to nitrogen are often the product of feedbacks between plant community composition, organic matter quality, and microbial decomposition processes. Together, these ecosystem properties may have led to the strong patterns we observed between soil C:N and the community composition of bacteria and fungi (Cheeke et al., 2016; Soares & Rousk, 2019).

Despite finding several linkages between structural diversity and the diversity of specific microbial community groups, the relationships between microbial community diversity and canopy structure were weaker than those observed for other environmental predictors. This may be due to weak connections between the aboveground and belowground traits of trees (Weemstra et al., 2016), or the inherent mismatch between the spatial scale of the canopy structure measurements and the scale at which we characterized soil microbial communities. It is well established that the magnitude and direction of diversity patterns in ecological relationships can vary with spatial scale (Rollinson et al., 2021; Wiens, 1989). Further, the relative importance of environmental drivers can be variable over space and time, and such variation can be hard to capture in their impact on ecological patterns at different scales (Wiens, 1989). The belowground dimensions of diversity change on a smaller spatial and temporal scale than the structural and species composition of forest canopies (Averill et al., 2019, 2021; Kivlin & Hawkes, 2020). Therefore, the linkages between above- and belowground components of ecosystems may become decoupled at increasingly large spatial scales (Martiny et al., 2011), possibly contributing to the large residual variance in microbial community composition in our study (Figure 4). For example, on a submeter scale, microbial richness may be impacted more strongly by soil properties, fine root activity, or individual host species traits rather than stand-level structure or biodiversity (Kivlin & Hawkes, 2016). In our data set, soil properties were measured at the same spatial scale of the microbial community (i.e., within a single soil core) and were therefore more closely matched in sampling spatial scale than the LiDAR or forest inventory data, likely contributing to the higher degree of association between microbial community composition and soil conditions compared with vegetation properties.

Further, the species richness and structural diversity of the herbaceous layer may be more influential than the forest canopy in shaping microbial richness and diversity (Chen et al., 2021; Yin et al., 2016). These understory plants may be particularly important in determining the community composition of mycorrhizal guilds, given that the mycorrhizal associations of canopy vegetation in temperate forests often do not match those of the understory plant species, which typically associate with either AM or ericoid mycorrhizal fungi (Ward et al., 2022; Wurzburger & Hendrick, 2009). Future work with terrestrial laser scanning or drones would be better suited to investigate these potential linkages, as measuring the herbaceous structural diversity is not currently possible with aerial LiDAR data due to constraints with data resolution and occlusion by the outer canopy (Li et al., 2021).

We investigated how forest structural diversity relates to soil microbial diversity within the central hardwoods region, but it is yet unclear how structural diversity may be linked to microbial community composition and richness in other forest types or biomes. Across broad temperature and moisture gradients, abiotic filtering, rather than structural diversity, may limit microbial community richness in forest soils (Nottingham et al., 2018). Soil properties, particularly pH, seem to be a ubiquitous predictor of microbial diversity (Davison et al., 2021; Tedersoo et al., 2014; van der Linde et al., 2018) and are often connected to plant community composition (Finzi et al., 1998; Templer et al., 2005) and changes in temperature and moisture conditions (Seaton et al., 2021),

but the connections between the drivers of above- and belowground diversity are still largely unexplored (Fei et al., 2022). In order to understand whether remote sensing of structural diversity could be used at broad scales to understand microbial diversity patterns, it is necessary to establish the biogeography of these relationships.

CONCLUSIONS

Our results indicate that LiDAR-derived structural diversity metrics measured at the stand level within the central hardwood region may be useful for predicting EM fungal richness as well as general shifts in microbial community composition in forest soils. Specifically, we suggest that forests with different degrees of structural diversity are likely to also differ in soil microbial community composition, and that a higher degree of canopy complexity supports greater EM fungal richness. Finally, we suggest that, of the four microbial guilds examined here, EM fungi are the best candidates for estimating community richness using remotely sensed canopy structure data. These patterns highlight the potential for using remote sensing for ecosystem monitoring, particularly in restoration research where microbial community composition may be used to achieve targeted ecosystem functions.

AUTHOR CONTRIBUTIONS

Ashley K. Lang and Elizabeth A. LaRue wrote the first draft of the manuscript. Ashley K. Lang, Elizabeth A. LaRue, and Stephanie N. Kivlin conducted the analyses. Ashley K. Lang, Elizabeth A. LaRue, Joey Gallion, Richard P. Phillips, Nicole Kong, and Stephanie N. Kivlin collected the data. All authors contributed to writing and editing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and R code for statistical analyses (Lang, 2023) are available from Zenodo: https://doi.org/10.5281/zenodo.8407741. Microbial sequences are available from NCBI: https://www.ncbi.nlm.nih.gov/bioproject/1026130.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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