RESEARCH ARTICLE

GCB-BIOENERGY
BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY

WILEY

Soil amendment with biochar and manure alters wood stake decomposition and fungal community composition

Ruirui Zhao[1] | Yong Liu[1] | Michael J. Gundale[2]

[1]Key Laboratory for Silviculture and Conservation, Ministry of Education, Beijing Forestry University, Beijing, China

[2]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden

Correspondence
Yong Liu, Key Laboratory for Silviculture and Conservation, Ministry of Education, Beijing Forestry University, Beijing 100083, China.
Email: lyong@bjfu.edu.cn

Michael J. Gundale, Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE 901-83 Umeå, Sweden.
Email: michael.gundale@slu.se

Abstract

Biochar and manure can be used for sustainable land management. However, little is known about how soil amendments might affect surface and belowground microbial processes and subsequent wood decomposition. In a split-split-split plot design, we amended soil with two rates of manure (whole plot; 0 and $9\,Mg\,ha^{-1}$) and biochar (split plot; 0 and $10\,Mg\,ha^{-1}$). Wood stakes of three species (hybrid poplar, triploid *Populus tomentosa* Carr.; aspen, *Populus tremuloides* Michx.; and pine, *Pinus taeda* L.) were placed in two positions (horizontally on the soil surface, and inserted vertically in the mineral soil), which served as a substrate for fungal growth. In 3 years, the decomposition rate (density loss), moisture content, and fungal community (via high-throughput sequencing methods) of stakes were evaluated. Results indicated that biochar and/or manure increased the wood stake decomposition rates, moisture content, and operational taxonomic unit abundance. However, the richness and diversity of fungi were dependent on wood stake position (surface > mineral), species (pine > the two *Populus*), and sample dates. This study highlights that soil amendment with biochar and/or manure can alter the fungal community, which in turn can enhance an important soil process (i.e., decomposition).

KEYWORDS
organic fertilizer, soil process, wood-colonizing fungal community

1 | INTRODUCTION

Forest soils have a large role in the global carbon (C) cycle because they harbor a substantial fraction of global soil C. Subsequently, key soil processes (e.g., organic C formation, C mineralization, nutrient transformations, and organic matter decomposition) in forests have implications for C sequestration and climate change (Jiang et al., 2020; Nottingham et al., 2020; Yu et al., 2022). Soil organic matter (SOM) is an important aspect of soil quality

(Bhattacharyya et al., 2022; Jaroszewicz et al., 2021) by increasing the water-holding capacity (Williams et al., 2016), reducing erosion (Feng et al., 2016), limiting soil compaction (Somerville et al., 2020), and serving as a substrate for microbial activity (Whalen et al., 2022). However, frequent harvesting operations or land-use changes can cause C loss, resulting in nutrient imbalances, drought, and reduced ecosystem services that soils provide (e.g., food and fiber), especially in plantations (Coban et al., 2022; Mishra et al., 2022; Newbold et al., 2015). Increasing SOM

has proven to be an effective measure in restoring fundamental physical, chemical, and biological soil properties (Krupek et al., 2022; Page-Dumroese et al., 2021) and enhancing soil health.

Biochar, which can transform labile C into a more stable form (Lehmann et al., 2012), has attracted worldwide attention for its capability to promote C sequestration and storage (Chagas et al., 2022; Pingree et al., 2022), which can serve to mitigate climate change (Hagenbo et al., 2022). In addition, biochar can enhance soil health (Gundale et al., 2016) by improving soil microbiome structure and function (Bahram et al., 2018; Xu et al., 2020), thus resulting in increased underground C cycling and above-ground productivity. Organic fertilizer amendment (e.g., manure and composts) is one of the most effective measures for sustainable land management, both in agriculture and forestry (Reardon, 2014; Williams, 1942; Yang et al., 2022), and its role in improving soil quality (e.g., physicochemical properties, structure, and microbiome functions), replenishing SOM loss, and minimizing land degradation has been well accepted (Augarten et al., 2023; Jin et al., 2022; Li et al., 2023).

In addition to C inputs from vegetation or organic amendments, microbial decomposition is another key factor that controls soil C content (Jastrow et al., 2007). The decomposition of organic matter in forests, both above and belowground, is a function of soil properties (e.g., temperature, moisture, nutrient availability, and microbial composition), substrate quality (e.g., nitrogen [N], lignin content, and C:N; Middleton et al., 2021; Risch et al., 2022; Viotti et al., 2021), and any soil condition changes at occur as a result of forest soil management practices (Piaszczyk et al., 2022; Tie et al., 2022). Generally, high organic matter decomposition rates are a sign of high soil quality and support higher forest productivity (Wang et al., 2010), and thus decomposition can serve as a useful response index of forest management activities. Biochar and/or organic amendments could alter organic matter decomposition through positive or negative priming effects (Joseph et al., 2021; Yu et al., 2020; Zheng et al., 2021), and by altering the soil microbial community composition or abundance (Minamino et al., 2019; Prayogo et al., 2013). However, the large variation in decomposition substrate qualities investigated among published studies makes it difficult to distinguish to what extent such decomposition responses originate from the soil by soil amendments or forest management practices.

The decomposition of "standardized" trembling aspen (*Populus tremuloides* Michx.) and pine (*Pinus taeda* L.) wood stakes with different qualities (e.g., C, N, lignin, and lignin:N) can help hold organic matter quality constant, and thus allow for the isolation of soil abiotic (e.g., moisture, temperature, and nutrient availability) and biotic

(e.g., microbial biomass, diversity, and function) changes caused by forest management practices (Finér et al., 2016; Risch et al., 2022). Much work has been done using the decay of wood stakes as an index of soil changes after land management (e.g., organic matter removal, and soil compaction) (Adams et al., 2021; Page-Dumroese, Jurgensen, et al., 2021). Especially, through the degradation of hybrid poplar (triploid *Populus tomentosa* Carr.), aspen, and pine stakes, our previous work indicated that biochar and/or manure amendment treatments accelerated wood decomposition and altered nutrient fluxes within 6 months in a hybrid poplar plantation (Zhao et al., 2022). However, how these amendments would affect wood decomposition and wood-inhabiting fungi over longer time scales remains unknown, which is crucial for the development of further forest soil management policies using biochar and/or organic fertilizers (Yang et al., 2021).

As a result, we aimed to investigate wood stake decomposition (density loss) and subsequent fungal community responses to soil manure and biochar amendments, both on the soil surface and belowground, over a 3-year period. Wood stakes of three species (hybrid poplar, trembling aspen, and pine) were placed horizontally on the soil surface (hereinafter, surface stakes) and inserted vertically in the 0–20 cm mineral soil (hereinafter, mineral stakes) to serve as an index indicating soil functioning and quality changes. These three species of wood stakes encompass a range of lignin, C, and N concentrations, which could favor the development of different wood-decomposing microbial communities (Wang et al., 2020). We hypothesized that biochar and/or manure would: (1) increase wood stake decomposition both on the soil surface and in the mineral soil; (2) alter the fungal community richness and abundance due to changed soil moisture; and further, (3) wood stake species and position would also be important factors affecting response to soil amendments.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

This 3-year field experiment was conducted in Guan County, Liaocheng City, Shandong Province, China (36°31′21″ N, 115°21′37″ E, 46 m above sea level), in the floodplain of the previous channel of the Yellow River left from the Western Han Dynasty (202 BC–8 AD). Since 1980, this region has been the focus of management activities aimed at preventing desertification through afforestation, and in the decade prior to our study, the site was used for poplar seedling cultivation. The climate at the study site is semiarid continental, and the average monthly temperature and precipitation are in Figure 1.
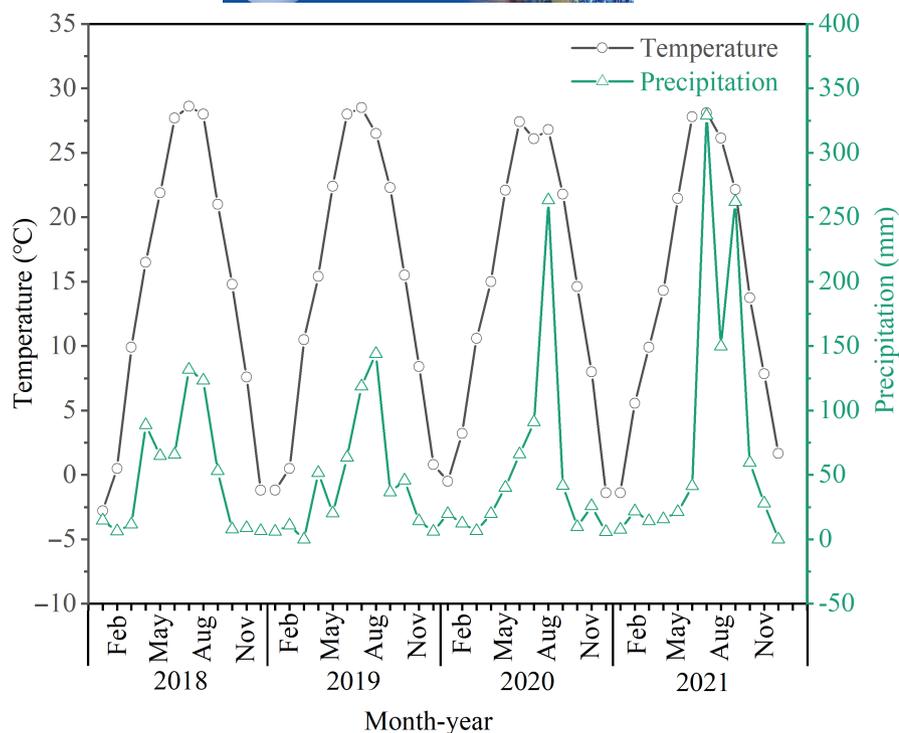
FIGURE 1 Monthly average air temperature and precipitation in the study site. The data set is provided by the Geographic remote sensing ecological network platform (www.gisrs.cn).

TABLE 1 Initial properties of sandy loam soil, maize straw biochar, pig manure, and hybrid poplar, aspen, and pine stakes (Zhao et al., 2022).

| Properties | 0–30 cm soil | Soil amendments | | Wood stakes | | |
		Maize straw biochar	Pig manure	Hybrid poplar	Aspen	Pine
pH	8.2	8.8	7.9	-	-	-
N (mg g^{-1})	2.20	10.70	14.64	1.40	1.10	0.80
P (mg g^{-1})	1.40	1.60	19.52	-	-	-
K (mg g^{-1})	8.30	2.60	26.84	-	-	-
C (%)	1.10	47.56	-	46.75	46.40	48.62
H (%)	-	2.80	-	-	-	-
C:N	5.00	44.00	-	354.64	421.81	631.65
Specific surface area (m^2 g^{-1})	-	5.0	-	-	-	-
Pore volume (cm^3 g^{-1})	-	6.93×10^{-3}	-	-	-	-
Pore size (nm)	-	5.60	-	-	-	-
Organic matter content (g kg^{-1})	7	-	450	-	-	-
Lignin (%)	-	-	-	20.92	20.64	31.09
Carbohydrate (%)	-	-	-	67.00	68.66	59.60
Lignin:N	-	-	-	149.43	189.50	403.72

Note: N, P, and K indicate total nitrogen, phosphorus, and potassium; C, carbon; H, hydrogen; -, not determined.

Soil (0–30 cm) in this study is sandy loam (sand:silt:clay is 62.3%:35.0%:2.7%). Additional details on soil properties are presented in Table 1.

We employed four soil treatments set up in a split-split-split plot experimental design. The first factor, manure (M), was applied at the whole plot level, with two rates: 0 (M0) and 9 (M9) Mg ha^{-1}. The second factor, biochar (B) was set up as a split-plot treatment with two levels, 0 (B0) and 10 (B10) Mg ha^{-1}. These manure and biochar treatments resulted in four soil treatment combinations (M0B0, M0B10, M9B0, and M9B10), each replicated three times, resulting in 12 plots in total, each 24 × 24 m squares separated with at least 8-m-wide buffer strips. In addition to soil amendment treatments, a third experimental factor was applied

as a split-split plot factor, which consisted of wood stakes that were installed in each of the two positions within each plot (horizontally on the soil surface, and vertically within the mineral soil). Furthermore, wood stakes were installed for each of three wood species (hybrid poplar, aspen, and pine), which served as a split-split-split plot factor.

2.2 | Soil amendments and wood stakes

Our experiment utilized biochar made from maize via slow pyrolysis (450–500°C for 2 h in an oxygen-limited rotary furnace; Qin Feng Zhong Cheng Biomass New Material Corporation), and pig manure used was supplied by Run Dong Fertilizer Corporation. The properties of these soil amendments are described in Table 1.

Kiln-dried and knot-free sapwood was selected for stake production, in which two surface (15 cm long) and two mineral (20 cm long) stakes of each species were cut from 2.5 cm × 2.5 cm stakes of 40 and 50 cm lengths, respectively, with the middle stake (10 cm) kept as a control (time = 0) to determine initial wood properties (Table 1). Before installation, one end of each mineral stake was treated with a wood sealer to avoid moisture loss (Jurgensen et al., 2006).

2.3 | Soil amendment implementation, wood stake installation, sampling, and calculations

After applying manure and biochar treatments to plot surfaces in April 2018, all soil treatments (including the un-amended M0B0) were mixed into the top 20 cm of soil using a rotary tiller (1GQN- 200; Weifang Sheng Xuan Machinery Corporation). After that, plots were planted with 1-year-old hybrid poplar (triploid "Beilinxiongzhu1" [*P. alba* × *P. glandulosa*] × [*P. tomentosa* × *P. bolleana*]) seedlings, with a 3 m × 4 m spacing; however, we note that tree growth was not the focus of our current study.

Regarding wood stake treatments, in July 2018, after removing the debris from the surface of forest land, 10 stakes of each species were placed on the soil surface (each 30 cm apart) and secured with a stainless-steel landscape staple. Using a 2.5 × 2.5 cm coring tool, we first created 10 holes per wood species (each 20 cm deep and about 30 cm apart) which helped reduce compaction from stake installation, mineral wood stakes were then inserted into the holes with the sealed end level with the soil surface. For more details, see Wang et al. (2019). Totally, 720 wood stakes were deployed (4 soil treatments × 3 replicates × 2 positions [surface and mineral] × 3 tree species [hybrid poplar, aspen, and pine] × 10 individual stakes).

In July 2020 and 2021—2 and 3 years after stakes were installed—five surface and five mineral stakes of each species were collected from each plot (a total of 720 stake samples, 4 soil treatments × 3 replicates × 2 stake positions × 3 tree species × 5 stakes × 2 sample dates). Wood stake samples were immediately weighed in the field after adhering material was removed from their surfaces, and then kept on ice as they were delivered to the laboratory.

Upon return from the field, wood shavings from each stake was collected for fungal DNA analysis within 24 h. First, both ends of each stake were cleaned with a sterile razor blade, then each end of the stake was drilled with sterile drill bits, and the two shaving samples of each stake were composited into one individual sample. All fungal samples were placed in 2 mL strip tubes and were frozen at −80°C until DNA extraction.

Subsequently, 10 days after wood stakes were placed in a climate chamber (65% relative humidity and 20°C), wood stake three dimensions (length × width × height) were measured. Wood stake density loss was calculated by subtracting the final from the initial wood density (g cm^{-3}) and expressed as a persent (Cline et al., 2017; Shorohova et al., 2008). After that, wood stakes were dried for 72 h at 105°C, and weighed for moisture content calculation (Zhao et al., 2022).

2.4 | DNA extraction, polymerase chain reaction amplification, and Illumina MiSeq sequencing

Microbial community genomic DNA was extracted using the FastDNA® Spin Kit for soil (MP Biomedicals) according to the manufacturer's instructions. The extracted DNA concentration and purity were determined using a UV–vis spectrophotometer (NanoDrop 2000; Thermo Scientific), and the DNA quality was evaluated by 1% agarose gel electrophoresis.

The primer pairs fITS7F (5′-GTGARTCATCGAATCT TTG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) were used to amplify the hypervariable ITS2 region, and the amplifications were performed on an ABI GeneAmp® 9700 PCR thermocycler (ABI) under the following programs: (1) initial denaturation at 95°C for 3 min, (2) 35 cycles of denaturing at 95°C for 30 s, (3) annealing at 55°C for 30 s, and (4) extension at 72°C for 45 s, single extension at 72°C for 10 min, ending at 10°C. polymerase chain reaction (PCR) reactions of each sample were performed in triplicate. Furthermore, the PCR products were extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega).

Purified amplicons were pooled in equimolar, and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. The raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA899460).

2.5 | Statistical analysis

For wood stake density loss and moisture content, we analyzed wood stake position effects first, then data were analyzed separately by position to see the main effects of manure, biochar, and wood species. Similarly, for wood stake fungal composition, we analyzed wood stake position effects firstly to see the overall changes, and then the effects of manure and biochar soil treatment combination, wood species, and/ or sample date were analyzed separately for each wood stake position.

2.5.1 | Wood stake decomposition and moisture content

After taking all factors (manure, biochar rates, wood stake position, and species) into consideration, and using density loss and moisture content as dependent variables for four-factor linear mixed effect (LME) models, three-factor LME models were used with manure, biochar, and wood stake species as independent factors and the density loss and moisture content as dependent variables. When the three-factor analysis revealed significant differences between treatment factors, we then performed post hoc Tukey's pairwise comparisons. Subsequently, we adopted two-factor LME models with manure and biochar as independent factors and the wood stake density loss and moisture content of each species at each position as dependent variables. When significant interactions among manure and biochar were detected, post hoc comparisons between the four soil treatments were conducted.

All LME models were conducted using the lmerTest package (Kuznetsova et al., 2017) in R version 4.1.1 (R Core Team, 2021), in which type III tests of fixed effects were used to examine main effects and interactions. The post hoc Tukey's pairwise comparisons were conducted using the emmeans package (Lenth et al., 2022). Furthermore, Pearson correlation analyses were used to study the correlations between wood stake density loss and moisture content for each species and position with all soil treatments combined. Scatter and bar charts were completed by Origin Pro 2022 (OriginLab).

2.5.2 | Fungal data processing

The raw ITS2 gene sequencing reads were de-multiplexed, quality-filtered by Fastp (version 0.20.0, https://github.com/OpenGene/fastp) (Chen et al., 2018) and merged by Flash (version 1.2.7, http://www.cbcb.umd.edu/software/flash) (Magoc & Salzberg, 2011) with the following criteria: (1) the reads were truncated to obtain an average quality score <20, and shorter sequence reads (truncated length <50 bp) were discarded; (2) raw reads containing ambiguous bases were discarded; and (3) only overlapping sequences >10 bp were assembled according to the overlapped sequences, and un-assembled reads were unwanted. The filtered reads were clustered into different operational taxonomic units (OTUs) with 97% similarity (Edgar, 2013; Stackebrandt & Goebel, 1994) by UPARSE (version 7.1, http://drive5.com/uparse/) (Edgar, 2013). The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al., 2007) against the Unite database 8.0 (http://unite.ut.ee/) using the confidence threshold of 0.70.

The analysis of fungal data was completed by R version 4.1.1 (R Core Team, 2021), which was mainly divided into the following parts. First, the MOTHUR program (version 1.30.2) was employed to conduct the rarefaction analysis, and Venn diagrams were used to demonstrate the number of unique OTUs in each group. Then we obtained the alpha-diversity indices (Chao and Shannon) through UPARSE (version 11) and utilized the Wilcoxon rank-sum test to check the differences between groups. Subsequently, fungal community bar charts were employed to see the overall structure at different groups at the genus level, and the principal coordinate analysis based on weighted-unifrac distance was applied to characterize microbial beta diversity. Permutational multivariate ANOVA (PERMANOVA) was performed by the Adonis function to show the difference between groups. Furthermore, the Wilcoxon rank-sum and Kruskal–Wallis rank tests were conducted to show the relative abundances of the top 15 genera different between groups. Finally, Pearson's correlations between the dominant genera and wood stake density loss, and moisture content were displayed using heatmaps, in which the third-year density loss and moisture were unpublished data.

3 | RESULTS

3.1 | Wood stake decomposition and moisture content

In our present study, the density loss and moisture content were investigated 2 years after wood stake installation.

Overall, wood stakes decomposed faster in the mineral soil (average, 52.33% density loss) than on the soil surface (average, 21.73% density loss) with all other factors combined ($F = 105.93$, $p < 0.001$). On the soil surface, biochar and wood species were the main factors affecting wood stake density loss, and interaction effects among manure, biochar, and wood species also existed (Table S1). Biochar application significantly increased wood stake density loss (average, 26.40%) with other factors combined (Figure 2a), and hybrid poplar and aspen stakes decomposed faster as compared to pine (27.12%, 24.81%, and 9.08%, respectively, Figure 2b). As for each wood species, biochar had significant effects on the density loss of three species, while interactions among manure and biochar were reflected in hybrid poplar and aspen stakes (Table 2). As compared to no biochar application, $10 \, \text{Mg ha}^{-1}$ biochar significantly increased the decomposition of hybrid poplar, aspen, and pine stakes (Figure 2c,e,g). In addition, biochar alone or combined with manure (M0B10 and M9B10) significantly increased the decomposition of surface hybrid poplar and aspen stakes as compared to the untreated M0B0 (Figure 2d,f).

In the mineral soil, wood species instead of manure or biochar was the main factor that affected wood stake decomposition (Table S1), in which significantly higher density loss was found in hybrid poplar and aspen stakes as compared to pine stakes (65.50%, 66.30%, and 28.40%, respectively) (Figure 2h). For each species, aspen and pine stake density loss was only affected by biochar amendment while the density loss of hybrid poplar stakes was unaffected by soil amendments (Table 2), in which biochar amendment significantly increased aspen and pine stake decomposition as compared to no biochar addition (Figure 2i,j).

Wood stake moisture content can reflect the soil water conditions about 2 weeks before sampling (Zhao et al., 2022). Generally, wood stakes had significantly less moisture on the soil surface (average, 46.67%) as compared to the mineral stakes (average, 68.97%) with other factors combined ($F = 65.55$, $p < 0.01$). On the soil surface, wood species instead of manure and biochar amendment was the main factor affecting wood stake moisture content (Table S1), in which significantly higher moisture content was detected in pine (58.50%) as compared to hybrid poplar (40.50%) and aspen stakes (41.00%) (Figure 3a). As for each wood species, hybrid poplar and aspen stake moisture

content was significantly affected by biochar with pine stake moisture content unaffected by soil amendments (Table 2), in which biochar amendment significantly increased the moisture content of hybrid poplar and aspen stakes as compared to no biochar addition (Figure 3b,c). Furthermore, the moisture content of surface hybrid poplar ($r = 0.722$, $p < 0.0000$), aspen ($r = 0.500$, $p = 0.0010$), and pine ($r = 0.493$, $p = 0.0012$) was positively correlated with the density loss with all the factors combined.

In the mineral soil, biochar amendment, and wood species were the main factors affecting wood stake moisture content (Table S1), in which higher wood stake moisture content was detected in soil treated with biochar (Figure 3d), and the highest moisture content was detected in aspen (79.20%) followed by hybrid poplar (72.10%) and pine (55.60%) (Figure 3e). Similarly, for each species, hybrid poplar, and aspen stake moisture content was notably affected by biochar amendment while the moisture content of pine stakes was not affected (Table 2), in which biochar addition significantly increased the moisture content of hybrid poplar and aspen stakes (Figure 3f,g). Similarly, positive correlations between moisture content and density loss were detected in hybrid poplar ($r = 0.505$, $p = 0.0009$), aspen ($r = 0.630$, $p = 0.0000$), and pine ($r = 0.570$, $p = 0.0001$) stakes.

3.2 | Wood stake fungal communities

3.2.1 | The alpha-diversity responses

A total of 24,070,074 fungal sequences were recovered after quality filtering (average 90,489 per sample), representing 1881 fungal OTUs at 97% sequence similarity. All of the fungal OTUs were classified into 11 different phyla, 39 classes, 102 orders, 234 families, 515 genera, and 874 species. Rarefaction curves for Shannon and Chao indices approached a plateau for most samples (Figure S1), which indicated that the sequences were adequate to represent the entire fungal population in wood stakes, allowing meaningful comparison between treatments.

At the OTU level, 719 and 410 OTUs, representing 38.22% and 21.80% of the total amount of OTUs, were exclusively represented in the mineral and surface stakes, respectively (Figure S2a). For surface stakes,

FIGURE 2 Surface wood stake density loss as affected by the main effect of biochar (a) and species (b); surface hybrid poplar stake density loss as affected by the main effects of biochar (c), and the interaction of manure and biochar (d); aspen stake density loss as affected by biochar (e), and the interactions of manure and biochar (f); pine stake density loss as affected by biochar (g); mineral wood stake density loss as affected by wood species (h) with soil treatments combined; aspen (i) and pine stake (j) density loss as affected by the main effect of biochar. Data are presented as means ± SE, and different letters indicate significant differences at $p \leq 0.05$.
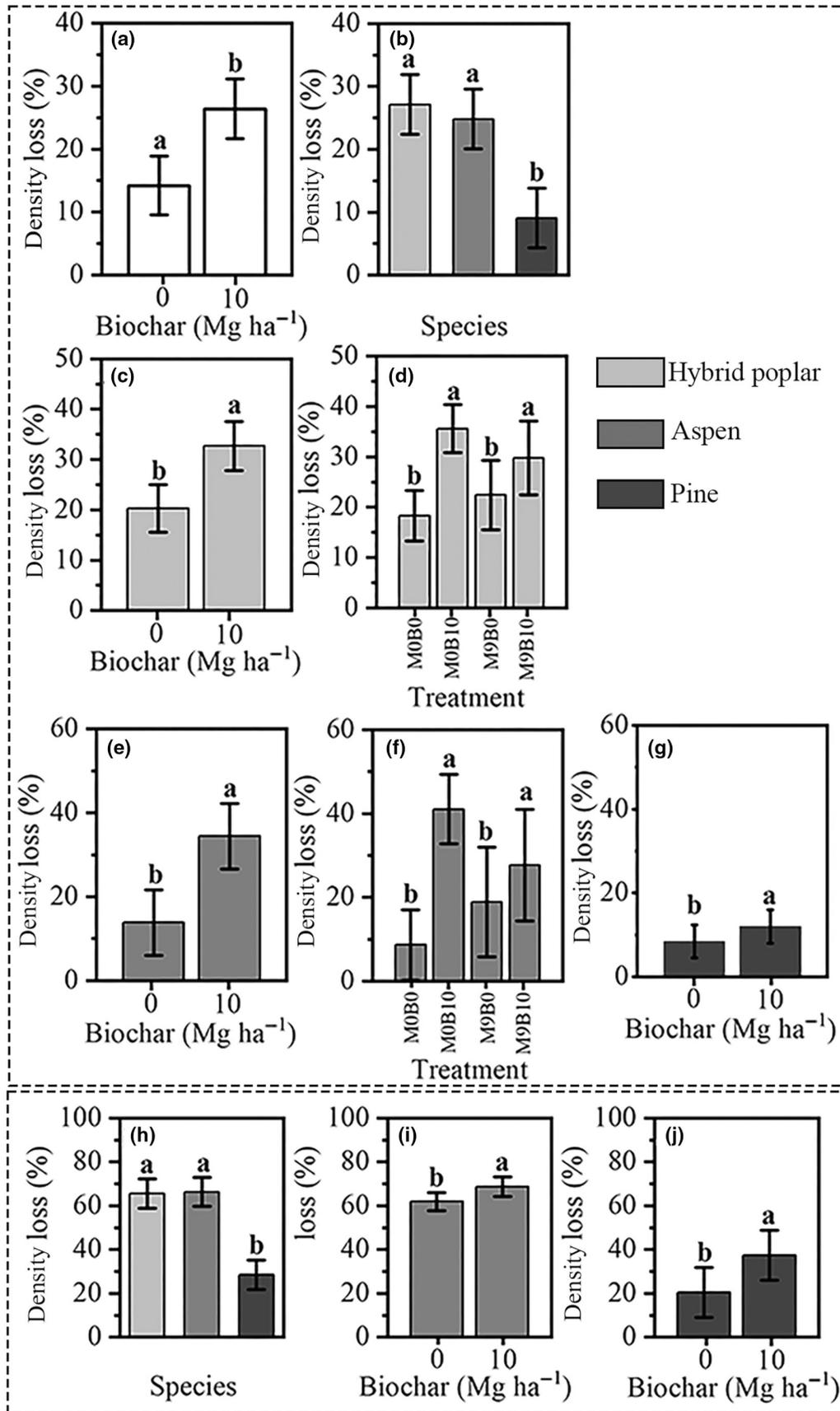
TABLE 2 Surface and mineral hybrid poplar, aspen, and pine stake density loss and moisture content responses to soil manure, biochar amendments, and their interactions after 2 years of decomposition.

Position and species	Source of variation	Density loss			Moisture content		
		df	F	p Values	df	F	p Values
Surface							
Hybrid poplar	Manure (M)	1	0.02	0.9002	1	6.75	0.9936
	Biochar (B)	1	38.96	0.0000***	1	0.00	0.0136*
	M × B	1	6.19	0.0180*	1	2.99	0.0927
Aspen	Manure (M)	1	0.01	0.9139	1	6.44	0.5542
	Biochar (B)	1	95.04	0.0000***	1	0.44	0.0157*
	M × B	1	30.94	0.0000***	1	3.16	0.0840
Pine	Manure (M)	1	0.13	0.7402	1	0.33	0.6566
	Biochar (B)	1	11.12	0.0021**	1	0.23	0.5722
	M × B	1	0.68	0.4147	1	0.61	0.4397
Mineral							
Hybrid poplar	Manure (M)	1	0.01	0.9217	1	34.91	0.4444
	Biochar (B)	1	3.57	0.0670	1	0.77	0.0000***
	M × B	1	1.92	0.1750	1	2.19	0.1478
Aspen	Manure (M)	1	1.62	0.2968	1	16.74	0.3305
	Biochar (B)	1	5.54	0.0242*	1	1.31	0.0002***
	M × B	1	1.03	0.3165	1	2.00	0.1656
Pine	Manure (M)	1	0.26	0.6466	1	0.61	0.6911
	Biochar (B)	1	20.84	0.0001***	1	0.19	0.4407
	M × B	1	0.12	0.7337	1	0.03	0.8679

Note: *, **, and *** denote significant effects at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

manure addition decreased (Figure S2b) while biochar had little effect on the abundance of OTUs (Figure S2c). Furthermore, stakes in soil treated with M0B10 had the most abundant and exclusive OTUs as compared to the other three treatments (Figure S2d). As for wood species, more OTUs and exclusive OTUs were found in pine stakes as compared to hybrid poplar and aspen (Figure S2e).

However, in the mineral soil, both manure and biochar application increased the OTU abundances of stakes (Figure S2f,g). Stakes in soil treated with 10 Mg ha^{-1} biochar (M0B10) had the most abundant OTUs while the most exclusive OTUs were detected in the high rate of manure and biochar (M9B10) (Figure S2h). Similarly, pine stakes had the most abundant and exclusive OTUs as compared to hybrid poplar and aspen (Figure S2i). However, a similar abundance of OTUs was detected after 2 and 3 years of decomposition (Figure S2j).

Instead of manure, biochar, and soil treatments, wood stake position, species, and sample date were the main factors affecting fungal richness and diversity expressed by the Chao and Shannon index (Figure 4). In general, fungal communities of surface stakes were significantly more abundant than stakes in the mineral soil (Figure 4a). Both on the soil surface and in the mineral soil, the highest richness and diversity of OTUs were detected in pine followed by hybrid poplar and aspen stakes, without significant differences between hybrid poplar and aspen stakes (Figure 4e,i). Furthermore, more fungal taxa were involved in the second than the third year of mineral stake decomposition (Figure 4j).

3.2.2 | Fungal community structure and beta diversity responses

Overall, fungal communities at the genus level strongly clustered according to wood stake positions (Figure 5a). For surface stakes, significant differences were evident with the manure treatment, but biochar had no effect on wood stake fungal communities (Figure 5b,c), and all manure and biochar combinations did not change the fungal communities significantly as compared to the untreated M0B0 (Figure 5d). In addition, pine stake fungal communities were significantly different from hybrid poplar and aspen (Figure 5e).
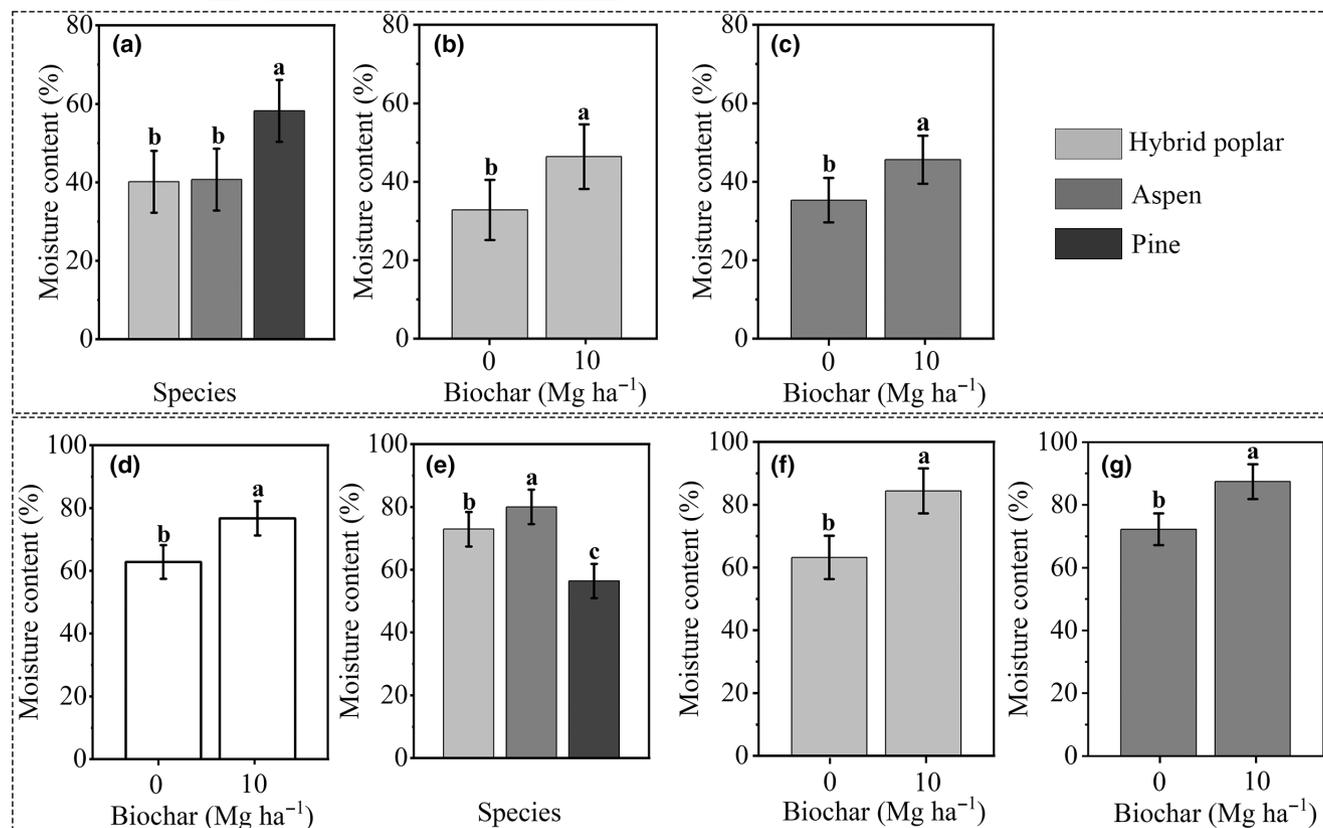
FIGURE 3 Surface wood stake moisture content as affected by the main effect of species (a); surface hybrid poplar (b) and aspen (c) stake moisture as affected by the main effects of biochar; mineral stake moisture content as affected by the main effects of biochar (d) and species (e) with soil treatments combined; mineral aspen (f) and pine (g) stake moisture content as affected by the main effects of biochar. Data are presented as means $\pm$ SE, and different letters indicate significant differences at $p \leq 0.05$.

However, in the mineral soil, manure and biochar application had no significant effects on the fungal communities of wood stakes (Figure 5f,g), while soil treated with the high rate of both manure and biochar (M9B10) significantly changed the fungal communities of stakes as compared to the M0B0, M0B10, and M9B0 (Figure 5h). Similarly, mineral pine stake fungal communities were significantly different from hybrid poplar and aspen (Figure 5i), but the sample date did not change the fungal communities significantly with other factors combined (Figure 5j).

Initial testing of genus-level effects for trial treatments (manure, biochar, soil treatments, wood stake position, species, and sample date) was presented in Figure S3, which provided identification that represents >1% of the total community. And the relative abundance differences of the top 15 genera among groups are shown in Figures 6 and 7 and Figure S4. Wood stake position changed the fungal taxa, in which only seven genera were shared for both surface and mineral stakes (Figure S3a). Surface stakes harbored a higher abundance of *Preussia*, *unclassified_c_Dothideomycetes*, *Coprinellus*, and *Coniochaeta* than stakes in the mineral soil, while the abundance of *unclassified_o_Sordariales* was the opposite (Figure S4).

Small differences were observed between the manure treatments, in which only *unclassified_f_Chaetomiaceae* (5.55%) and *unclassified_f_Xylariaceae* (4.05%) were exclusive in soil treated with manure, and *Trichoderma* (4.05%) and *Talaromyces* (3.01%) were exclusive in soil without manure application (Figure S3b). For surface stakes, the abundance of all the top five genera was significantly higher in soil treated with manure (Figure 6a). However, in the mineral soil, manure amendment significantly increased the abundance of *unclassified_f_Chaetomiaceae*, but less *Hydnophlebia*, *Exophiala*, *Trichoderma*, and *unclassified_c_Dothideomycetes* were enriched as compared to no manure addition (Figure 7a).

Similarly, biochar had few effects on measured fungal community structure, in which *unclassified_f_Chaetomiaceae* (4.76%), *Talaromyces* (2.36%), and *Phaeoacremonium* (1.90%) were exclusive in soil treated with biochar, and *Psathyrella* (3.42%), *unclassified_f_Xylariaceae* (2.62%), and *Lasiodiplodia* (2.62%) were exclusively detected in soil without biochar (Figure S3c). For stakes on the soil surface, the top five genera were less clustered in soil treated with biochar (Figure 6b). In the mineral soil, however, biochar application decreased the
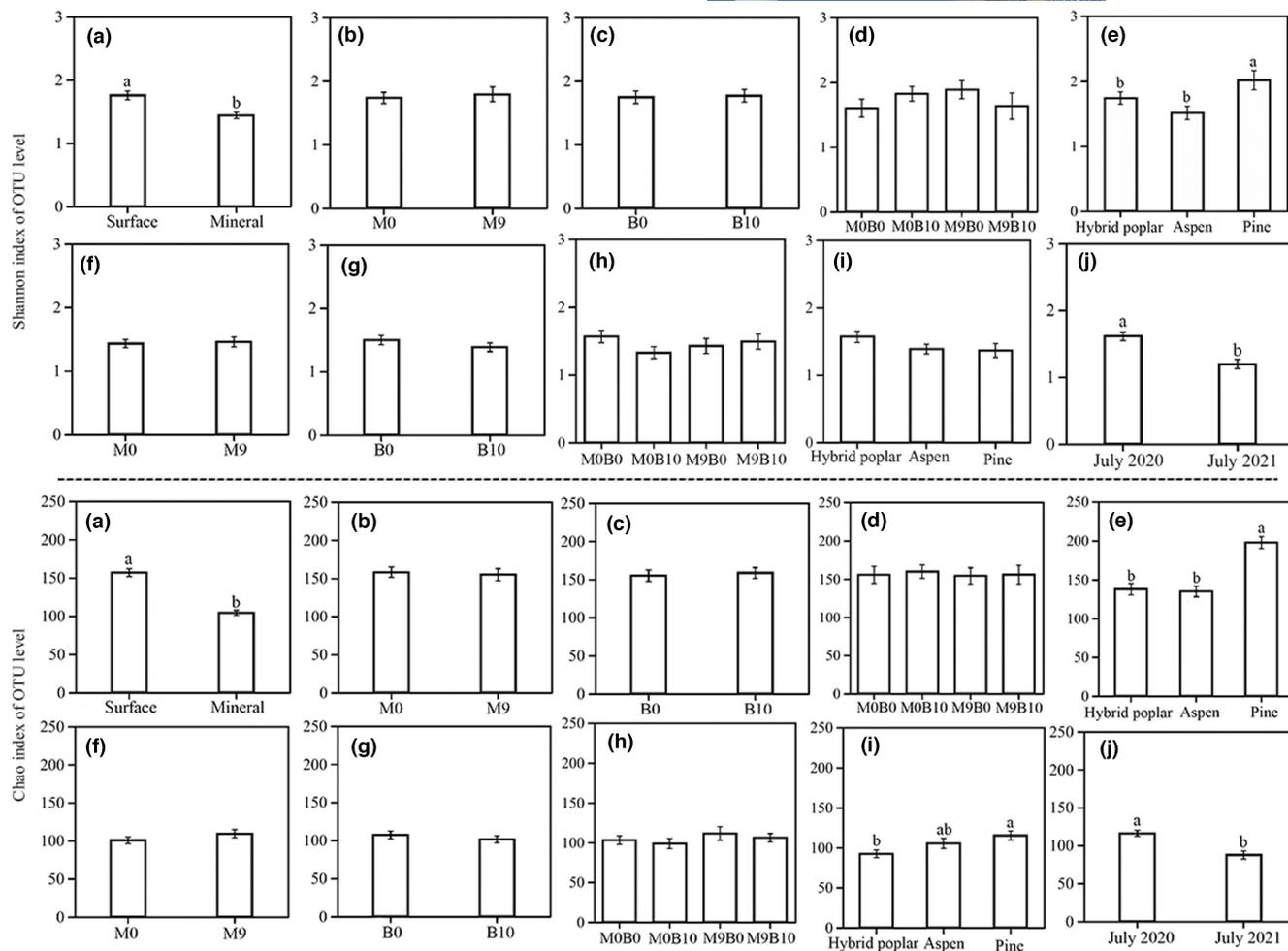
FIGURE 4 Wood stake fungal diversity (Shannon, upper) and richness (Chao, lower) indices of different groups. (a) Represents wood stakes at different positions; (b–e) represent surface stakes of different manure rates (b), biochar rates (c), biochar and manure combinations (d), and wood stake species (e); and (f–j) represent mineral stakes of different manure rates (f), biochar rates (g), biochar and manure combinations (h), wood stake species (i), and sample dates (j). Wilcoxon rank-sum test was performed. Data are presented as means $\pm$ SE, different letters indicate significant differences at $p \leq 0.05$, and bars without letters are not significantly different.

abundance of the top four genera but increased the abundance of *Phaeoacremonium* (Figure 7b).

For the four soil treatments, a total of seven shared genera were detected in all four treatments while the most unique taxa were found in M9B10 (Figure S3d). On the soil surface, all soil treatments increased the abundance of *unclassified_o_Pleosporales* but decreased the abundance of *unclassified_o_Xylariales* as compared to the untreated M0B0. The highest abundance of *Hypoxylon* and *Coniochaeta* was detected in M0B10, and the most abundant *Paecilomyces* was in M9B0 (Figure 6c). In the mineral soil, all soil treatments increased the abundance of *Scytalidium* but decreased the abundance of *Exophiala*. The abundance of *unclassified_o_Sordariales* and *Hydnophlebia* was decreased in M9B10 but increased in M0B10 and M9B0 as compared to M0B0 (Figure 7c). Meanwhile, M9B0 increased while M0B10 and M9B10

decreased the abundance of *unclassified_o_Hypocreales* as compared to the M0B0 (Figure 7c).

The fungal taxa composition of the hybrid poplar and aspen stakes was similar (seven shared genera), while the most unique genera were found in pine stakes (Figure S3e). On the soil surface, *Preussia* and *Hypoxylon* were most abundant in hybrid poplar followed by aspen and pine stakes, and the abundance of *Coprinellus* and *unclassified_c_Dothideomycetes* was highest in aspen followed by hybrid poplar and pine stakes. However, *Coniochaeta* was significantly more abundant in pine stakes (Figure 6d). In the mineral soil, *unclassified_o_Sordariales*, *unclassified_o_Hypocreales*, and *Exophiala* were significantly abundant in hybrid poplar or aspen as compared to pine stakes, while the abundance of *Hydnophlebia* was highest in pine followed by aspen and hybrid poplar stakes, and the most
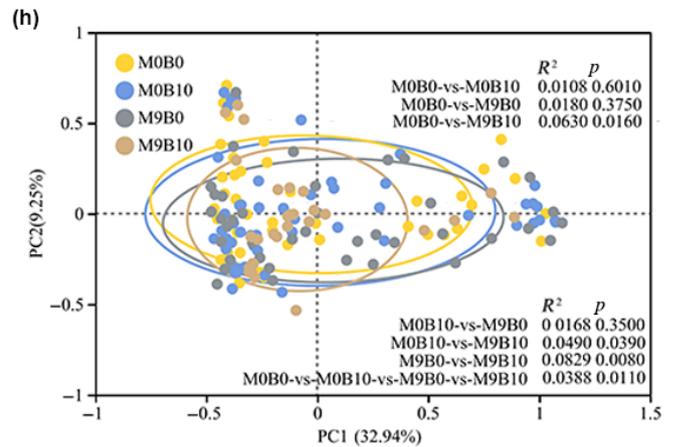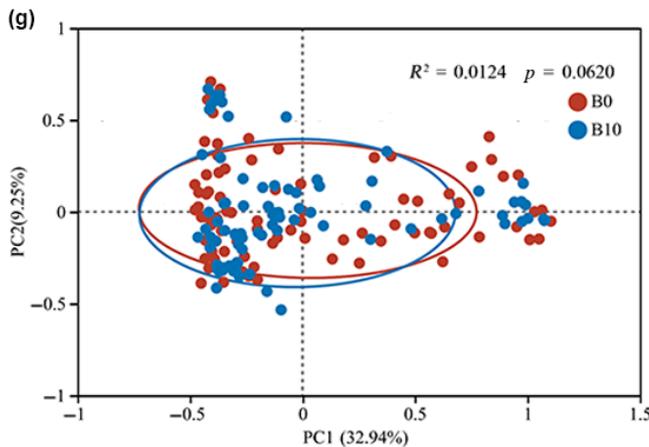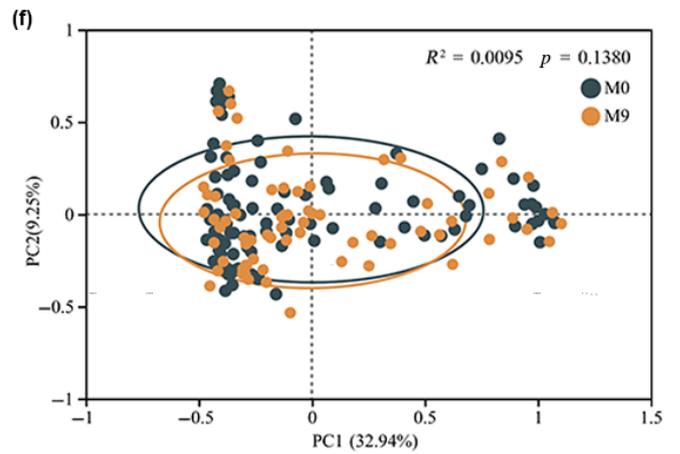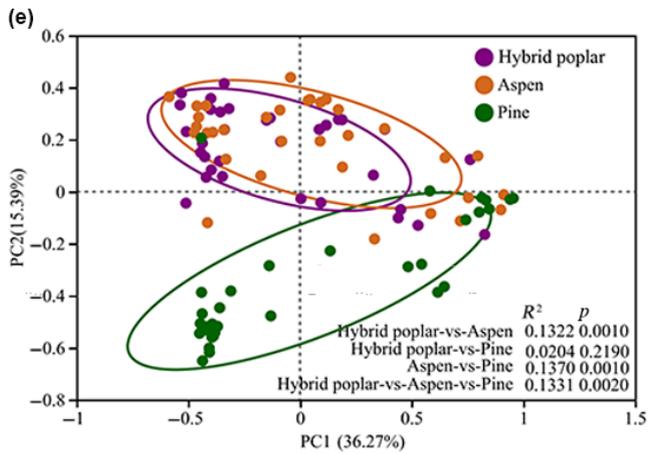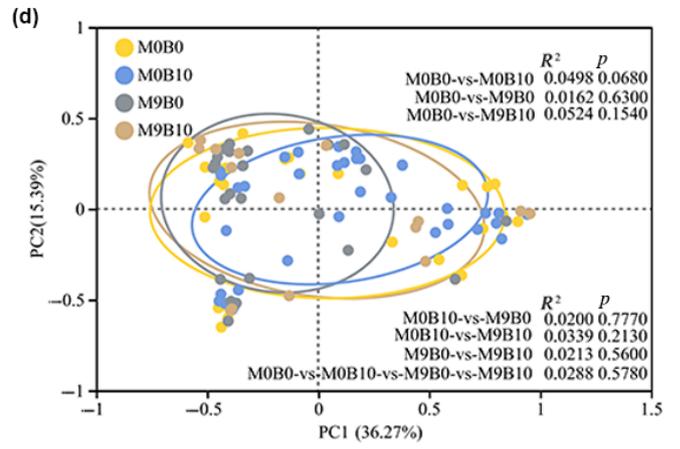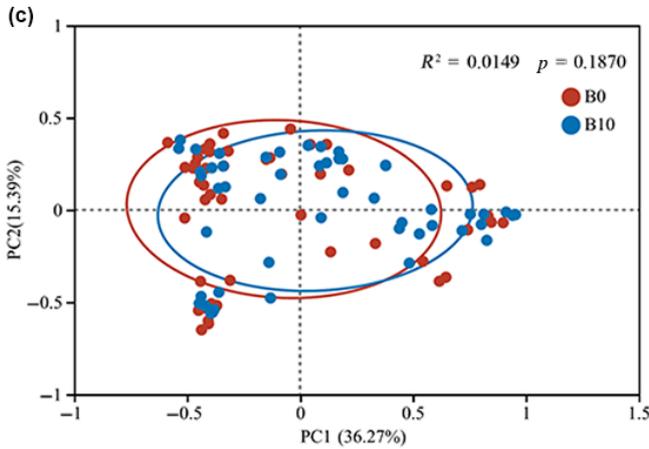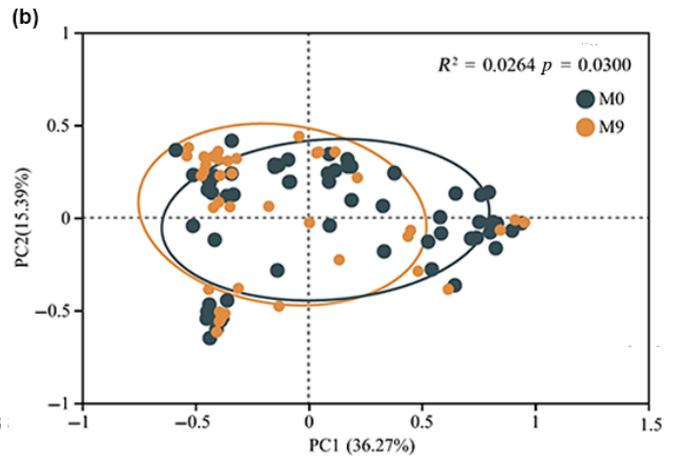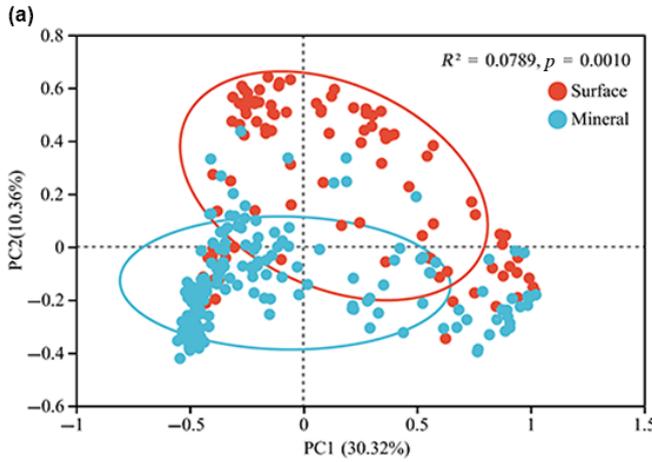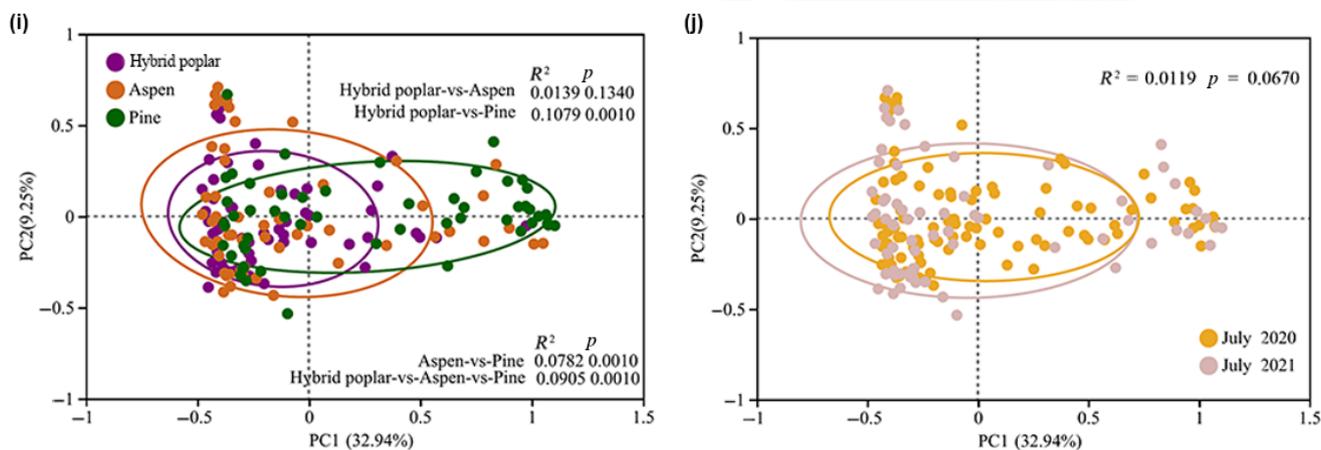
FIGURE 5 Principal coordinate analysis on genus level based on weighted-unifrac distance of all stakes at different groups. (a) Represents wood stakes at different positions; (b–e) represent surface stakes of different manure rates (b), biochar rates (c), biochar and manure combinations (d), and wood stake species (e); and (f–j) represent mineral stakes of different manure rates (f), biochar rates (g), biochar and manure combinations (h), wood stake species (i), and sample dates (j). Permutational multivariate ANOVA (PERMANOVA) tests were performed by the Adonis function to show the difference between groups.

abundant *Scytalidium* was detected in hybrid poplar followed by pine and aspen stakes (Figure 7d).

Sample date also affected the top 15 abundant fungal genera composition of mineral stakes, in which the top 3 abundant fungal genera were *Hydnophlebia* (14.69%), *unclassified_o_Sordariales* (8.24%), and *Preussia* (7.71%) 2 years after wood stakes were installed, while in the third year, the top 3 genera were *unclassified_o_Sordariales* (14.50%), *Hydnophlebia* (12.15%), and *unclassified_f_ Chaetomiaceae* (8.59%) (Figure S3f). Furthermore, significantly more abundant *Hydnophlebia*, *Trichoderma*, *Psathyrella*, and *Preussia* were detected after 2 years of decomposition, while in the third year, *unclassified_f_ Chaetomiaceae* was more enriched (Figure 7e).

3.3 | Relationships between wood stake decomposition, moisture content, and fungal community

To better understand the relationship between wood stake decomposition and fungi community, Pearson correlation analysis was conducted for three wood species at each position (Figure 8). For surface stakes, both the density loss and moisture content of hybrid poplar stakes were significantly correlated with several genera of *Cystobasidiomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Sordariomycetes*, *Tremellomycetes*, and *Agaricomycetes* class, in which more positive correlations were detected (Figure 8a). Similarly, aspen stake density loss and moisture content were significantly related to genera of *Sordariomycetes*, *Dothideomycetes*, *Agaricomycetes*, and *Eurotiomycetes* class (Figure 8b). However, pine stake density loss was more related to fungal taxa as compared to

the moisture content, in which genera of *Agaricomycetes*, *Sordariomycetes*, *Dothideomycetes*, *Eurotiomycetes*, and *unclassified_p_Ascomycota* class were involved (Figure 8c).

In the mineral soil, hybrid poplar and aspen stake density loss and moisture content were only significantly correlated with several genera of *Sordariomycetes*, *Dothideomycetes*, *Agaricomycetes*, *unclassified_p_ Ascomycota*, and *Eurotomycetes* class, while more genera of *Sordariomycetes*, *unclassified_k_Fungi*, *Eurotiomycetes*, *Agaricostilbomycetes*, *Dothideomycetes*, and *Cystobasidiomycetes* class were significantly related to the density loss and moisture of pine stakes (Figure 8d–f).

4 | DISCUSSION

We hypothesized that biochar and manure would increase wood stake decay and alter the richness and abundance of fungal communities, and that such effects would depend upon wood species and positions. Biochar and/or manure indeed accelerated the decomposition rates and increased the moisture content of wood stakes both on the soil surface and in the mineral soil. Furthermore, soil amendments increased the OTU abundance in wood stakes, especially in the mineral soil, but the fungal richness and diversity were more affected by wood stake position, wood species, and sampling dates.

4.1 | Wood stake decomposition and moisture content

Biochar can enhance soil moisture content, especially in coarse-textured soils, because of its high porosity
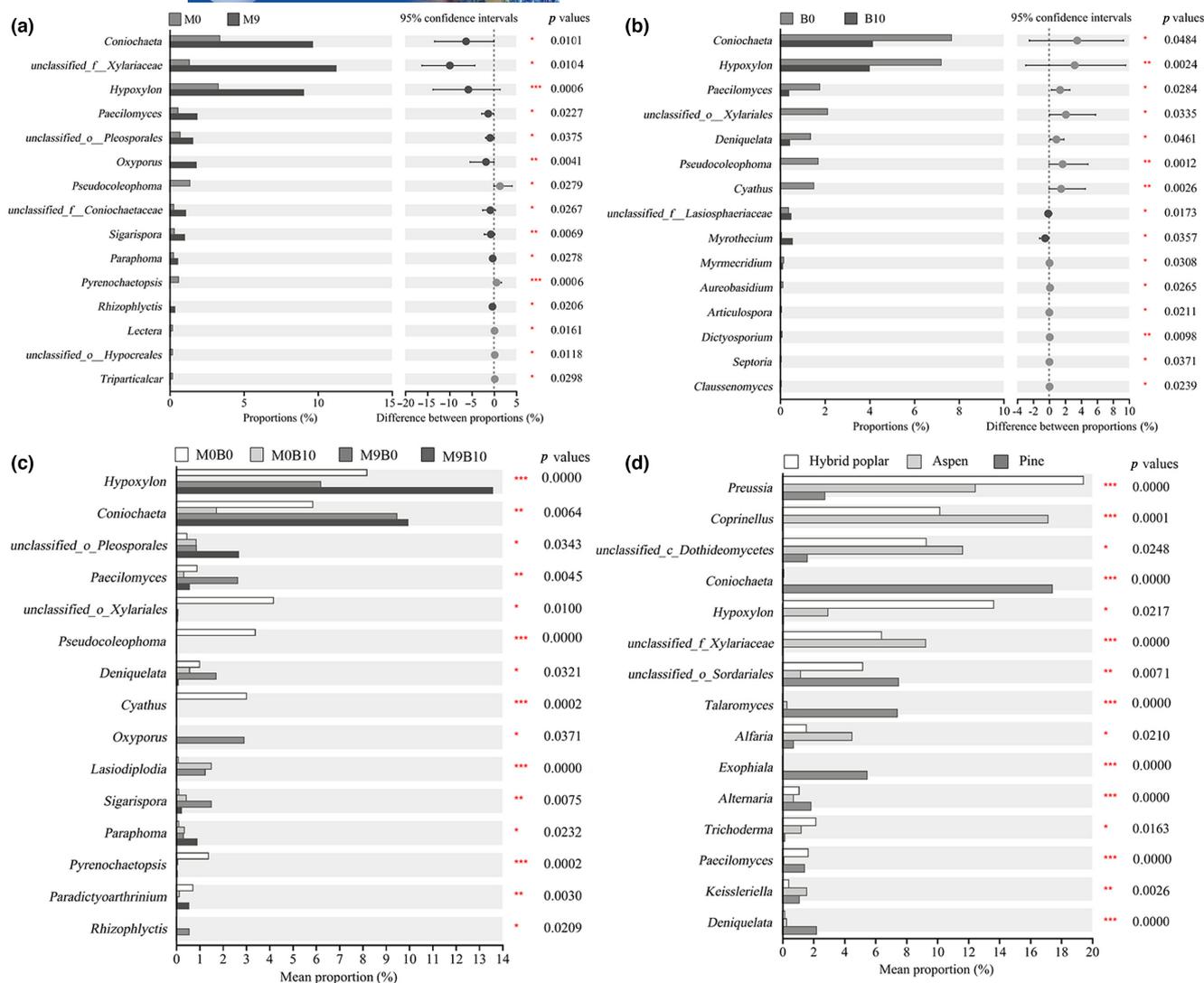
FIGURE 6 Relative abundances of the top 15 genera in surface wood stakes showed significant differences among manure (a), biochar (b), manure and biochar combination treatments (c), and wood stake species (d). Comparisons between multiple and two groups were conducted using the Wilcoxon rank-sum and Kruskal–Wallis rank test, respectively. In multiple comparisons, Welch's post hoc tests were used. *, **, and *** indicate significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

(Razzaghi et al., 2020). Similarly, in our current study, biochar at $10\,Mg\,ha^{-1}$ significantly increased the density loss and moisture content of stakes both on the soil surface and in the mineral soil (Figures 2 and 3). However, manure hardly affected wood stake decomposition and moisture content except combined with biochar (Figures 2 and 3). This may be because the longer longevity of biochar compared to manure may have caused longer-lasting positive effects (Seyedsadr et al., 2022; Zhao et al., 2022). Furthermore, there may have been synergistic positive effects of compost–biochar mixtures on the water-storage capacity in the sandy, low-organic content soils (Liu et al., 2012; Seyedsadr et al., 2022), and moisture uptake from the surrounding soil matrix may have served as an important control on decomposition (Gora et al., 2019;

Seyedsadr et al., 2022). In addition, manure or other soil amendments can alter microbial functions and subsequent wood decomposition by changing soil nutrient availability (Pingree et al., 2022), however, in our previous study, we did not find soil N availability in soil treated with manure (Zhao et al., 2022), which may explain why we found no main effects of the manure treatment on decomposition in the current study.

On the soil surface, soil amendments increased the density loss of three wood species, but the increased moisture content was only detected in the *Populus* species. Meanwhile, mineral pine stake density loss was increased by soil amendments without moisture content changes. These inconsistent changes indicate that soil biotic (e.g., fungal community) rather than abiotic (e.g.,
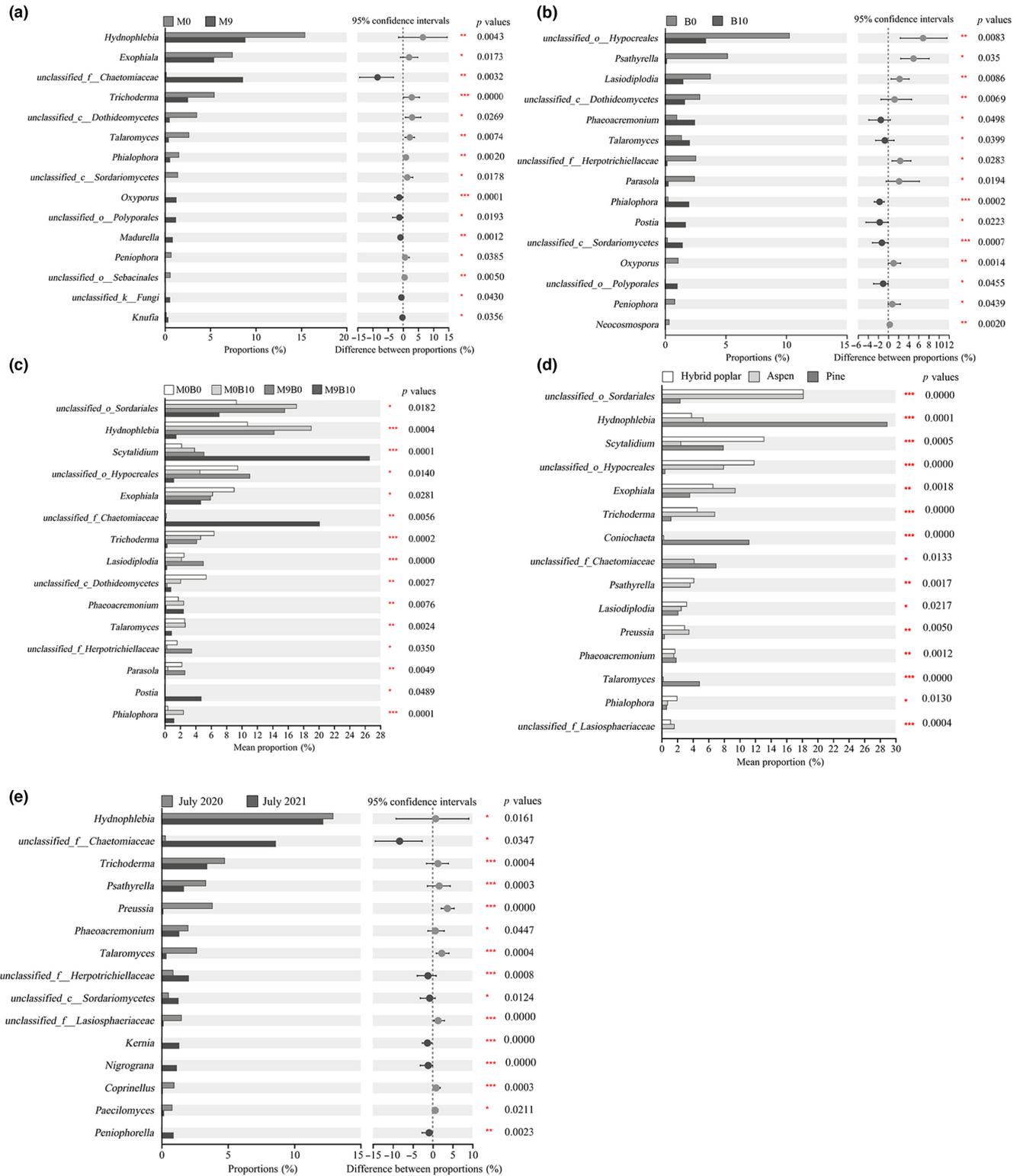
FIGURE 7 Relative abundances of the top 15 genera of mineral stakes showed significant differences among manure (a), biochar (b), manure and biochar combination treatments (c), species (d), and sample date (e). Comparisons between multiple and two groups were conducted using the Wilcoxon rank-sum and Kruskal–Wallis rank test, respectively. In multiple comparisons, Welch's post hoc tests were used. *, **, and *** indicate significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

temperature and moisture content) changes caused by soil amendments were responsible for the responses in wood decomposition we observed because biochar can cause preferential adsorption of wood and litter-degrading fungi in organic matter-poor sandy loam soil (Anasonye et al., 2018), which may cause degradation changes.

FIGURE 8 Pearson correlation heatmap of wood stake density loss, moisture content, and the top 50 abundant genera of fungi in surface hybrid poplar (a), aspen (b), and pine (c), and mineral hybrid poplar (d), aspen (e), and pine (f).

4.2 | Soil amendment, wood stake position, and species effects on wood stake fungal community

4.2.1 | Fungal alpha and beta diversity

Biochar and/or manure could indirectly increase the richness and diversity of fungi related to wood degradation

and litter decomposition through nutrient supply (Pingree et al., 2022) or amelioration of soil pH (Han et al., 2022; Tarin et al., 2021). Although soil manure, biochar, and their combinations had no significant impacts on the fungal richness and diversity of wood stakes at the OTU level (Figure 4), fungal composition at the genera level indeed significantly clustered according to soil amendments (Figure 5). This finding is consistent with data presented

by Zhang et al. (2021), where soil manure or biochar amendments caused soil micro-environmental changes.

Higher species richness, in fungal ecology, is commonly thought to relate to higher rates of nutrient cycling and decomposition via additive or synergistic benefits of the fungal niche complementarity (van der Wal et al., 2013). However, consistent with results reported by Wang et al. (2020), we found surface stakes had higher fungal richness and diversity (Figure 4), but decomposed slower as compared to mineral stakes ($F = 105.93$, $p < 0.001$). Furthermore, pine stakes decayed slower even though had higher fungal richness and diversity as compared to the two *Populus* stakes in our present study (Figures 2 and 4). These patterns may be due to that poor soil conditions on the forest surface, and lower-quality pine stakes limit fungal growth rates, which may prevent species with fast colonization and growth traits from dominating substrates, and result in greater species coexistence with slower-growing fungal species (Maynard et al., 2017).

4.2.2 | Fungal composition and abundance

Wood-decaying fungi are divided into three main categories (brown-rot, white-rot, and soft-rot fungi) depending on their enzyme capabilities to degrade lignocellulose substrates. Soft-rot fungi (mostly *Ascomycete*) can degrade polysaccharides in the surface layers of plants, brown-rot fungi (*Basidiomycetes*) mainly degrade cellulose and hemicellulose and slightly modify lignin (Andlar et al., 2018; Sanchez, 2009; Viotti et al., 2021), and white-rot fungi (especially *basidiomycetes*) are able to decompose all the major polymers, for example, lignin, cellulose, and hemicellulose (Tuor et al., 1995). Although soil temperature and humidity are thought to be the main factors affecting saprotrophic fungi in specific forests, stakes placed in the mineral soil did not always have a higher fungal abundance at the genus level (Figure S4), suggesting other factors (e.g., oxygen, airborne spores, drought tolerance of fungi) also serve as important control of fungal diversity (Jiang et al., 2014; Li et al., 2022).

Soil manure amendment changed the abundance of the top 15 genera, which may relate to the altered wood stake moisture content in the third year of decomposition ($F = 5.75$, $p < 0.01$, not published data), or the improved microenvironment of microorganisms (Zhang et al., 2022). But the different response directions of the two stake positions (positive for surface stakes, but negative for mineral stakes; Figures 5 and 6) may have occurred because fungi prioritized the utilization of labile manure C in the mineral soil rather than the more complex wood substrate (Siedt et al., 2021). Biochar application, however, decreased the abundance of the top 15 fungal genera in both

surface and mineral soil wood stakes (Figures 5 and 6), which may be due to the relatively high alkalinity of the soil, biochar, and manure used in our study (pH = 8.2, 8.8, and 7.9 respectively), which was likely not conducive to the cellulose nonenzymatic deconstruction process, and may have also disrupted lignocellulose degradation by brown-rot fungi (Andlar et al., 2018).

Cellulose-degrading soft-rot fungi are believed to be the first decomposers of woody debris, followed by brown- and white-rot fungi in the middle stages of the wood decomposition (Rajala et al., 2015; Tarvainen et al., 2020). Furthermore, *Basidiomycete* species are thought to dominate over *Ascomycetes* during the later stages of decomposition (Duong et al., 2008) since they can synthesize the enzymes required to degrade complex polymers such as lignin (Deacon, 1997; Kodsueb et al., 2008). However, in our study, we found that *Ascomycetes* always dominated at the two sampling dates (75.19% and 79.62%, respectively, after 2 and 3 years of decomposition) with other factors combined, which may be because stakes were still at an early stage of decomposition after 2 years of installation (average density loss 27.30%), and the decay has not entered the later stages of decomposition, even in the third year (density loss average 52.50%, unpublished data). In addition, the most abundant 15 genera achieved their greatest abundance in mineral stakes decomposed for 2 years (sampled in July 2020), as compared to 3 years after installation (sampled in July 2021), which may relate to the low water content (average 33.93%, unpublished data), or that the remaining C was in the form of lignin, which is more recalcitrant (Fukasawa et al., 2009; Kurth et al., 2013).

5 | CONCLUSIONS

Using standard decomposition substrates (i.e., wood stakes) as an index of soil functioning, our study indicates that biochar alone or combined with manure increased the density loss and moisture content of stakes both on the soil surface and in the mineral soil. In addition, the application of biochar and/or manure increased the OTU abundances in wood stakes, especially in the mineral soil. But OTU richness and diversity were more strongly regulated by wood stake position, wood species, and sample dates. We proposed that 10 Mg ha^{-1} biochar should be taken into account by forest management operations aimed at enhancing or sustaining soil quality in plantations with similar soil conditions, and additional benefits can be achieved with the combined addition of 9 Mg ha^{-1} manure. Furthermore, the degradation of standard substrates can provide scientific data about soil process changes both on the soil surface and

belowground, which can be used to develop better forestry policy.

CONFLICT OF INTEREST STATEMENT

We declare that we authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author on reasonable request.

ORCID

Yong Liu https://orcid.org/0000-0002-6958-0205
Michael J. Gundale https://orcid.org/0000-0003-2447-609X

REFERENCES

Adams, M. B., Jurgensen, M., Palik, B., Miller, C., & Page-Dumroese, D. S. (2021). Wood stake decomposition twenty years after organic matter removal at the Lake States LTSP sites. *Forest Ecology and Management*, *496*, 119456. https://doi.org/10.1016/j.foreco.2021.119456

Anasonye, F., Tammeorg, P., Parshintsev, J., Riekkola, M.-L., & Tuomela, M. (2018). Role of biochar and fungi on PAH sorption to soil rich in organic matter. *Water, Air, & Soil Pollution*, *229*(2), 1–14. https://doi.org/10.1007/s11270-018-3708-2

Andlar, M., Rezic, T., Mardetko, N., Kracher, D., Ludwig, R., & Šantek, B. (2018). Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. *Engineering in Life Sciences*, *18*(11), 768–778. https://doi.org/10.1002/elsc.201800039

Augarten, A. J., Malone, L. C., Richardson, G. S., Jackson, R. D., Wattiaux, M. A., Conley, S. P., Radatz, A. M., Cooley, E. T., & Ruark, M. D. (2023). Cropping systems with perennial vegetation and livestock integration promote soil health. *Agricultural & Environmental Letters*, *8*(1), e20100. https://doi.org/10.1002/ael2.20100

Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., … Bork, P. (2018). Structure and function of the global topsoil microbiome. *Nature*, *560*(7717), 233–237. https://doi.org/10.1038/s41586-018-0386-6

Bhattacharyya, S. S., Ros, G. H., Furtak, K., Iqbal, H. M. N., & Parra-Saldívar, R. (2022). Soil carbon sequestration – An interplay between soil microbial community and soil organic matter dynamics. *Science of the Total Environment*, *815*, 152928. https://doi.org/10.1016/j.scitotenv.2022.152928

Chagas, J. K. M., de Figueiredo, C. C., & Ramos, M. L. G. (2022). Biochar increases soil carbon pools: Evidence from a global meta-analysis. *Journal of Environmental Management*, *305*, 114403. https://doi.org/10.1016/j.jenvman.2021.114403

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). *fastp*: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1101/274100

Cline, L. C., Schilling, J. S., Menke, J., Groenhof, E., & Kennedy, P. G. (2017). Ecological and functional effects of fungal endophytes on wood decomposition. *Functional Ecology*, *32*(1), 181–191. https://doi.org/10.1111/1365-2435.12949

Coban, O., De Deyn, G. B., & van der Ploeg, M. (2022). Soil microbiota as game-changers in restoration of degraded lands. *Science*, *375*(6584), abe0725. https://doi.org/10.1126/science.abe0725

Deacon, J. W. (1997). *Modern mycology* (3rd ed.). Blackwell Science.

Duong, L., McKenzie, E., Hyde, K., & Lumyong, S. (2008). Fungal succession on senescent leaves of *Castanopsis diversifolia* in Doi Suthep-Pui National Park, Thailand. *Fungal Diversity*, *30*, 23–36.

Edgar, R. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*(10), 996–998. https://doi.org/10.1038/nmeth.2604

Feng, Z., Leung, L. R., Hagos, S., Houze, R. A., Burleyson, C. D., & Balaguru, K. (2016). More frequent intense and long-lived storms dominate the springtime trend in central US rainfall. *Nature Communications*, *7*, 13429. https://doi.org/10.1038/ncomms13429

Finér, L., Jurgensen, M., Palviainen, M., Piirainen, S., & Page-Dumroese, D. S. (2016). Does clear-cut harvesting accelerate initial wood decomposition? A five-year study with standard wood material. *Forest Ecology and Management*, *372*, 10–18. https://doi.org/10.1016/j.foreco.2016.03.060

Fukasawa, Y., Osono, T., & Takeda, H. (2009). Microfungus communities of Japanese beech logs at different stages of decay in a cool temperate deciduous forest. *Canadian Journal of Forest Research*, *39*(8), 1606–1614. https://doi.org/10.1139/x09-080

Gora, E. M., Lucas, J. M., & Yanoviak, S. P. (2019). Microbial composition and wood decomposition rates vary with microclimate from the ground to the canopy in a tropical forest. *Ecosystems*, *22*(6), 1206–1219. https://doi.org/10.1007/s10021-019-00359-9

Gundale, M. J., Nilsson, M.-C., Pluchon, N., & Wardle, D. A. (2016). The effect of biochar management on soil and plant community properties in a boreal forest. *GCB Bioenergy*, *8*(4), 777–789. https://doi.org/10.1111/gcbb.12274

Hagenbo, A., Anton-Fernandez, C., Bright, R. M., Rasse, D., & Astrup, R. (2022). Climate change mitigation potential of biochar from forestry residues under boreal condition. *Science of the Total Environment*, *807*(Pt 3), 151044. https://doi.org/10.1016/j.scitotenv.2021.151044

Han, Z. Q., Xu, P. S., Li, Z. T., Lin, H. Y., Zhu, C., Wang, J. Y., & Zou, J. W. (2022). Microbial diversity and the abundance of keystone species drive the response of soil multifunctionality to organic substitution and biochar amendment in a tea plantation. *GCB Bioenergy*, *14*(4), 481–495. https://doi.org/10.1111/gcbb.12926

Jaroszewicz, B., Cholewińska, O., Chećko, E., & Wrzosek, M. (2021). Predictors of diversity of deadwood-dwelling macrofungi in a European natural forest. *Forest Ecology and Management*, *490*, 119123. https://doi.org/10.1016/j.foreco.2021.119123

Jastrow, J. D., Amonette, J. E., & Bailey, V. L. (2007). Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Climatic Change*, *80*(1–2), 5–23. https://doi.org/10.1007/s10584-006-9178-3

Jiang, M. K., Medlyn, B. E., Drake, J. E., Duursma, R. A., Anderson, I. C., Barton, C. V. M., Boer, M. M., Carrillo, Y., Castañeda-Gómez, L., Collins, L., Crous, K. Y., De Kauwe, M. G., Dos Santos, B. M., Emmerson, K. M., Facey, S. L., Gherlenda, A. N., Gimeno, T. E., Hasegawa, S., Johnson, S. N., ... Ellsworth, D. S. (2020). The fate of carbon in a mature forest under carbon dioxide enrichment. *Nature*, *580*(7802), 227–231. https://doi.org/10.1038/s41586-020-2128-9

Jiang, X. Y., Cao, L. X., Zhang, R. D., Yan, L. J., Mao, Y., & Yang, Y. W. (2014). Effects of nitrogen addition and litter properties on litter decomposition and enzyme activities of individual fungi. *Applied Soil Ecology*, *80*, 108–115. https://doi.org/10.1016/j.apsoil.2014.04.002

Jin, L., Jin, N., Wang, S. Y., Li, J. W., Meng, X., Xie, Y. D., & Yu, J. H. (2022). Changes in the microbial structure of the root soil and the yield of Chinese Baby Cabbage by chemical fertilizer reduction with bio-organic fertilizer application. *Microbiology Spectrum*, *10*(6), 1–14. https://doi.org/10.1128/spectrum.01215-22

Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., & Lehmann, J. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, *13*(11), 1731–1764. https://doi.org/10.1111/gcbb.12885

Jurgensen, M., Reed, D., Page-Dumroese, D. S., Laks, P., Collins, A., Mroz, G., & Degórski, M. (2006). Wood strength loss as a measure of decomposition in northern forest mineral soil. *European Journal of Soil Biology*, *42*(1), 23–31. https://doi.org/10.1016/j.ejsobi.2005.09.001

Kodsueb, R., McKenzie, E. H. C., Lumyong, S., & Hyde, K. D. (2008). Fungal succession on woody litter of *Magnolia liliifera* (Magnoliaceae). *Fungal Diversity*, *30*, 55–72.

Krupek, F. S., Redfearn, D., Eskridge, K. M., & Basche, A. (2022). Ecological intensification with soil health practices demonstrates positive impacts on multiple soil properties: A large-scale farmer-led experiment. *Geoderma*, *409*, 115594. https://doi.org/10.1016/j.geoderma.2021.115594

Kurth, V. J., Fransioli, N., Fulé, P. Z., Hart, S. C., & Gehring, C. A. (2013). Stand-replacing wildfires alter the community structure of wood-inhabiting fungi in southwestern ponderosa pine forests of the USA. *Fungal Ecology*, *6*(3), 192–204. https://doi.org/10.1016/j.funeco.2013.01.006

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lehmann, J., Czimczik, C., Laird, D., & Sohi, S. (2012). Stability of biochar in soils. In J. Lehmann & S. Joseph (Eds.), *Biochar for environmental management: Science, technology and implementation* (pp. 215–238). Routledge.

Lenth, R. V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2022). *emmeans: Estimated marginal means, aka least-squares means* (version 1.7.4-1). R package version 1.7.5. https://github.com/rvlenth/emmeans

Li, L., He, L. X., Li, Y. Q., Wang, Y. F., Ashraf, U., Hamoud, Y. A., & Pan, S. G. (2023). Deep fertilization combined with straw incorporation improved rice lodging resistance and soil properties of paddy fields. *European Journal of Agronomy*, *142*, 126659. https://doi.org/10.1016/j.eja.2022.126659

Li, T., Cui, L. Z., Song, X. F., Cui, X. Y., Wei, Y. L., Tang, L., & Xu, Z. H. (2022). Wood decay fungi: An analysis of worldwide research. *Journal of Soils and Sediments*, *22*(6), 1688–1702. https://doi.org/10.1007/s11368-022-03225-9

Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B., & Glaser, B. (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and Soil Science*, *175*, 698–707. https://doi.org/10.1002/jpln.201100172

Magoc, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*(21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Maynard, D. S., Bradford, M. A., Lindner, D. L., van Diepen, L. T. A., Frey, S. D., Glaeser, J. A., & Crowther, T. W. (2017). Diversity begets diversity in competition for space. *Nature Ecology & Evolution*, *1*(6), 156. https://doi.org/10.1038/s41559-017-0156

Middleton, T. E., McCombs, A. L., Gailans, S. R., Carlson, S., Karlen, D. L., Moore, K. J., & McDaniel, M. D. (2021). Assessing biological soil health through decomposition of inexpensive household items. *Applied Soil Ecology*, *168*(2), 104699. https://doi.org/10.1016/j.apsoil.2021.104099

Minamino, Y., Fujitake, N., Suzuki, T., Yoshitake, S., Koizumi, H., & Tomotsune, M. (2019). Effect of biochar addition on leaf-litter decomposition at soil surface during three years in a warm-temperate secondary deciduous forest, Japan. *Scientific Reports*, *9*(1), 16961. https://doi.org/10.1038/s41598-019-53615-2

Mishra, A., Humpenöder, F., Churkina, G., Reyer, C. P. O., Beier, F., Bodirsky, B. L., & Popp, A. (2022). Land use change and carbon emissions of a transformation to timber cities. *Nature Communications*, *13*(1), 4889. https://doi.org/10.1038/s41467-022-32244-w

Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., & Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, *520*(7545), 45–50. https://doi.org/10.1038/nature14324

Nottingham, A. T., Meir, P., Velasquez, E., & Turner, B. L. (2020). Soil carbon loss by experimental warming in a tropical forest. *Nature*, *584*(7820), 234–237. https://doi.org/10.1038/s41586-020-2566-4

Page-Dumroese, D. S., Jurgensen, M. F., Miller, C. A., Busse, M. D., Curran, M. P., Terry, T. A., & Murray, M. (2021). Decomposition of wood stakes in the Pacific Northwest after soil compaction

and organic matter removal. *Forest Ecology and Management*, *494*, 119362. https://doi.org/10.1016/j.foreco.2021.119362

Page-Dumroese, D. S., Sanchez, F. G., Udawatta, R. P., Perry, C. H., & González, G. (2021). Soil health assessment of forest soils. In D. Karlen, D. E. Stott, & M. M. Mikha (Eds.), *Soil health series* (Vol. *1*, pp. 100–138). John Wiley & Sons, Incorporation.

Piaszczyk, W., Lasota, J., Błońska, E., & Foremnik, K. (2022). How habitat moisture condition affects the decomposition of fine woody debris from different species. *Catena*, *208*, 105765. https://doi.org/10.1016/j.catena.2021.105765

Pingree, M. R. A., Kardol, P., Nilsson, M. C., Wardle, D. A., Maaroufi, N. I., & Gundale, M. J. (2022). No evidence that conifer biochar impacts soil functioning by serving as microbial refugia in boreal soils. *GCB Bioenergy*, *14*(8), 972–988. https://doi.org/10.1111/gcbb.12978

Prayogo, C., Jones, J. E., Baeyens, J., & Bending, G. D. (2013). Impact of biochar on mineralisation of C and N from soil and willow litter and its relationship with microbial community biomass and structure. *Biology and Fertility of Soils*, *50*(4), 695–702. https://doi.org/10.1007/s00374-013-0884-5

R Core Team. (2021). *R: A language and environment for statistical computing (version 4.1.1)*. R Core Team. http://www.R-project.org/

Rajala, T., Tuomivirta, T., Pennanen, T., & Mäkipää, R. (2015). Habitat models of wood-inhabiting fungi along a decay gradient of Norway spruce logs. *Fungal Ecology*, *18*, 48–55. https://doi.org/10.1016/j.funeco.2015.08.007

Razzaghi, F., Obour, P. B., & Arthur, E. (2020). Does biochar improve soil water retention? A systematic review and meta-analysis. *Geoderma*, *361*, 114055. https://doi.org/10.1016/j.geoderma.2019.114055

Reardon, S. (2014). Manure fertilizer increases antibiotic resistance. *Nature*. https://doi.org/10.1038/nature.2014.16081

Risch, A. C., Page-Dumroese, D. S., Schweiger, A. K., Beattie, J. R., Curran, M. P., Finér, L., & Jurgensen, M. F. (2022). Controls of initial wood decomposition on and in forest soils using standard material. *Frontiers in Forests and Global Change*, *5*, 829810. https://doi.org/10.3389/ffgc.2022.829810

Sanchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, *27*(2), 185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Seyedsadr, S., Sipek, V., Jacka, L., Snehota, M., Beesley, L., Pohorely, M., & Trakal, L. (2022). Biochar considerably increases the easily available water and nutrient content in low-organic soils amended with compost and manure. *Chemosphere*, *293*, 133586. https://doi.org/10.1016/j.chemosphere.2022.133586

Shorohova, E., Kapitsa, E., & Vanha-Majamaa, I. (2008). Decomposition of stumps in a chronosequence after clear-felling vs. clear-felling with prescribed burning in a southern boreal forest in Finland. *Forest Ecology and Management*, *255*(10), 3606–3612. https://doi.org/10.1016/j.foreco.2008.02.042

Siedt, M., Schäffer, A., Smith, K. E. C., Nabel, M., Roß-Nickoll, M., & van-Dongen, J. T. (2021). Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. *Science of the Total Environment*, *751*, 141607. https://doi.org/10.1016/j.scitotenv.2020.141607

Somerville, P. D., Farrell, C., May, P. B., & Livesley, S. J. (2020). Biochar and compost equally improve urban soil physical and biological properties and tree growth, with no added benefit in combination. *Science of the Total Environment*, *706*, 135736. https://doi.org/10.1016/j.scitotenv.2019.135736

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, *44*(4), 846–849. https://doi.org/10.1099/00207713-44-4-846

Tarin, M. W. K., Fan, L. L., Xie, D. J., Tayyab, M., Rong, J. D., Chen, L. Y., & Zheng, Y. S. (2021). Response of soil fungal diversity and community composition to varying levels of bamboo biochar in red soils. *Microorganisms*, *9*(7), 1385. https://doi.org/10.3390/microorganisms9071385

Tarvainen, O., Saravesi, K., Pennanen, T., Markkola, A.-M., Suokas, M., & Fritze, H. (2020). Fungal communities in decomposing wood along an energy wood harvest gradient. *Forest Ecology and Management*, *465*, 118070. https://doi.org/10.1016/j.foreco.2020.118070

Tie, L. H., Hu, J. X., Peñuelas, J., Sardans, J., Wei, S. Z., Liu, X., & Huang, C. D. (2022). The amounts and ratio of nitrogen and phosphorus addition drive the rate of litter decomposition in a subtropical forest. *Science of the Total Environment*, *833*, 155163. https://doi.org/10.1016/j.scitotenv.2022.155163

Tuor, U., Winterhalter, K., & Fiechter, A. (1995). Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay. *Journal of Biotechnology*, *41*, 1–17. https://doi.org/10.1016/0168-1656(95)00042-O

van der Wal, A., Geydan, T. D., Kuyper, T. W., & de Boer, W. (2013). A thready affair: Linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, *37*(4), 477–494. https://doi.org/10.1111/1574-6976.12001

Viotti, C., Bach, C., Maillard, F., Ziegler-Devin, I., Mieszkin, S., & Buee, M. (2021). Sapwood and heartwood affect differentially bacterial and fungal community structure and successional dynamics during *Quercus petraea* decomposition. *Environmental Microbiology*, *23*(10), 6177–6193. https://doi.org/10.1111/1462-2920.15522

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, S. L., Zhang, W. D., & Sanchez, F. (2010). Relating net primary productivity to soil organic matter decomposition rates in pure and mixed Chinese fir plantations. *Plant and Soil*, *334*, 501–510. https://doi.org/10.1007/s11104-010-0400-8

Wang, W. W., Lindner, D. L., Jusino, M. A., Page-Dumroese, D. S., Palmer, J. M., Banik, M. T., Jurgensen, M., Kymberly, D., & Liu, Y. (2020). Wood-colonizing fungal community response to forest restoration thinnings in a *Pinus tabuliformis* plantation in northern China. *Forest Ecology and Management*, *476*, 118459. https://doi.org/10.1016/j.foreco.2020.118459

Wang, W. W., Page-Dumroese, D. S., Jurgensen, M., Miller, C., Walitalo, J., Chen, X., & Liu, Y. (2019). Restoration thinning impacts surface and belowground wood decomposition. *Forest Ecology and Management*, *449*, 117451. https://doi.org/10.1016/j.foreco.2019.117451

Whalen, E. D., Grandy, A. S., Sokol, N. W., Keiluweit, M., Ernakovich, J., Smith, R. G., & Frey, S. D. (2022). Clarifying the evidence for microbial- and plant-derived soil organic matter, and the

path toward a more quantitative understanding. *Global Change Biology*, 28(4), 7167–7185. https://doi.org/10.1111/gcb.16413

Williams, A., Hunter, M. C., Kammerer, M., Kane, D. A., Jordan, N. R., Mortensen, D. A., & Davis, A. S. (2016). Soil water holding capacity mitigates downside risk and volatility in US rainfed maize: Time to invest in soil organic matter? *PLoS One*, 11(8), e0160974. https://doi.org/10.1371/journal.pone.0160974

Williams, G. B. (1942). Manufacture of fertilizer from urban wastes. *Nature*, 150(3801), 299. https://doi.org/10.1038/150299a0

Xu, W. H., Whitman, W. B., Gundale, M. J., Chien, C. C., & Chiu, C. Y. (2020). Functional response of the soil microbial community to biochar applications. *GCB Bioenergy*, 13(1), 269–281. https://doi.org/10.1111/gcbb.12773

Yang, Q., Zhou, H. W., Bartocci, P., Fantozzi, F., Mašek, O., Agblevor, F. A., & McElroy, M. B. (2021). Prospective contributions of biomass pyrolysis to China's 2050 carbon reduction and renewable energy goals. *Nature Communications*, 12(1), 1–12. https://doi.org/10.1038/s41467-021-21868-z

Yang, Y. H., Li, M. J., Wu, J. C., Pan, X. Y., Gao, C. M., & Tang, D. W. S. (2022). Impact of combining long-term subsoiling and organic fertilizer on soil microbial biomass carbon and nitrogen, soil enzyme activity, and water use of winter wheat. *Frontiers in Plant Science*, 12, 788651. https://doi.org/10.3389/fpls.2021.788651

Yu, Z., Ciais, P., Piao, S. L., Houghton, R. A., Lu, C. Q., Tian, H. Q., & Zhou, G. Y. (2022). Forest expansion dominates China's land carbon sink since 1980. *Nature Communications*, 13(1), 5374. https://doi.org/10.1038/s41467-022-32961-2

Yu, Z. Y., Ling, L., Singh, B. P., Luo, Y., & Xu, J. M. (2020). Gain in carbon: Deciphering the abiotic and biotic mechanisms of biochar-induced negative priming effects in contrasting soils. *Science of the Total Environment*, 746, 141057. https://doi.org/10.1016/j.scitotenv.2020.141057

Zhang, M. Y., Zhang, L., Riaz, M., Xia, H., & Jiang, C. C. (2021). Biochar amendment improved fruit quality and soil properties and microbial communities at different depths in citrus production. *Journal of Cleaner Production*, 292, 126062. https://doi.org/10.1016/j.jclepro.2021.126062

Zhang, X., Zhang, Q. Q., Zhan, L. P., Xu, X. T., Bi, R. Y., & Xiong, Z. Q. (2022). Biochar addition stabilized soil carbon sequestration by reducing temperature sensitivity of mineralization and altering the microbial community in a greenhouse vegetable field. *Journal of Environmental Management*, 313, 114972. https://doi.org/10.1016/j.jenvman.2022.114972

Zhao, R. R., Liu, Y., Page-Dumroese, D. S., Dumroese, R. K., & Wang, K. (2022). Enhancing soil quality of short rotation forest operations using biochar and manure. *Forests*, 13(12), 2090. https://doi.org/10.3390/f13122090

Zheng, T. H., Zhang, J., Tang, C. J., Liao, K. T., & Guo, L. P. (2021). Positive and negative priming effects in an Ultisol in relation to aggregate size class and biochar level. *Soil & Tillage Research*, 208, 104874. https://doi.org/10.1016/j.still.2020.104874

SUPPORTING INFORMATION

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