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RESEARCH ARTICLE

Biochar and manure additions increased above- and belowground wood decomposition, and soil enzyme activities in a sandy loam soil

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Abstract

While biochar and manure can provide considerable benefits to soil properties, how these amendments may alter soil microbial activity and decomposition processes remains unknown. In a split-split-split-plot experiment, we amended a sandy loam soil with three rates of manure (whole plot; 0, 3, 9 Mg ha^{-1}) and biochar (split-plot; 0, 2.5, 10 Mg ha^{-1}), and installed three species of wood stakes (split-split-split plot; triploid poplar, *Populus tomentosa* Carr.; trembling aspen, *Populus tremuloides* Michx.; and loblolly pine, *Pinus taeda* L.) on the soil surface and in the mineral soil (split-split plot) to serve as a substrate for microbial degradation. Wood stakes were sampled 3 years after installation to assess decomposition rates (mass loss), and changes in wood carbon (C) and nitrogen (N). In addition, soil extracellular enzyme activities at the 0–20 cm depth were examined. Biochar alone, especially 10 Mg ha^{-1}, increased wood stake decomposition and moisture content on the soil surface and in the mineral soil. Manure at the rate of 9 Mg ha^{-1} increased soil N-acetyl-β-D-glucosaminidase, α-glucosidase, and aryl sulfatase activities by 91%, 17%, and 48% respectively. Because of the synergistic benefits of biochar and manure, we suggest that, in this climatic regime and soil texture, 10 Mg ha^{-1} biochar can be used for soil C sequestration and soil quality improvement, and 9 Mg ha^{-1} manure can be used in combination with biochar to build soil organic matter in plantations.

KEYWORDS
microbial decay, organic fertilizer, poplar plantation, soil process

1 | INTRODUCTION

Soil organic matter (SOM) is critical for regulating plant available water and nutrients, supporting heterotrophic soil organisms, and reducing soil compaction, erosion, and other site stressors (Feng et al., 2016; Siedt et al., 2021). Together, these beneficial attributes can maintain soil quality and site productivity (Jurgensen

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et al., 1997; Vance, 2000) and the supply of food and fiber (Eisenstein, 2020; Marris, 2022). Forest soils can generally retain a large portion of the terrestrial carbon (C) pool (Rumpel, 2019). However, anthropogenic climate alterations (Prietzel et al., 2016), land management and use changes (Haghighi et al., 2010) as well as frequent harvesting operations (Labelle et al., 2022), especially in plantations, can deplete soil C, cause nutrient imbalances, and exacerbate drought conditions that result in a subsequent decrease in ecosystem functions (Coban et al., 2022; Mishra et al., 2022; Newbold et al., 2015). Adding organic matter has been proven an important measure in reducing soil C losses and improving soil quality and productivity (Han et al., 2020; Yu et al., 2021).

Biochar created from a variety of crop and forest residues is a recalcitrant form of C that enhances soil physiochemical properties to improve soil biological functions for decades to centuries (Amonette & Joseph, 2009; Li et al., 2022). Manure is another amendment that can be added to soil alone or in combination with biochar to increase SOM content (Zhang et al., 2023). Both biochar and manure can promote soil fertility, vegetation production, and stable aggregate formation (Rayne & Aula, 2020; Xu et al., 2023; Zhang et al., 2023) and can be used to increase soil C and organic matter levels in plantations. However, it is often difficult and expensive to continuously measure soil property changes associated with adding amendments (Janzen et al., 2021; Joseph et al., 2022); thus, other comprehensive and sensitive indicators are necessary to reflect forest management or soil amendment effects on soil quality (Middleton et al., 2021; Page-Dumroese, Jurgensen, et al., 2021; Page-Dumroese, Sanchez, et al., 2021).

In general, high organic matter decomposition rates on and in forest soils are generally linked to higher site and soil productivity (Mayer et al., 2023; Wang et al., 2010). Further, organic matter decomposition rates are a function of soil biotic and abiotic properties that can be altered by soil amendments (Mayer et al., 2023; Tie et al., 2022). For example, altered soil C or organic matter contents caused by soil amendments or other forest management can result in soil microbial process changes and subsequent decomposition rate and nutrient cycling alterations through the production of a variety of soil enzymes, for example, acid phosphatase, cellulase, α-glucosidase, β-glucosidase, N-acetyl-β-D-glucosaminidase, and aryl sulfatase (Mayer et al., 2023), because these enzymes mainly drive the degradation of lignin, cellulose, and hemicellulose of the substrates (Albiach et al., 2000; Fatemi et al., 2016; Zhang et al., 2018). Therefore, although soil organic amendment effects on soil microbial activities, for example, decomposition, is difficult to distinguish due to the various substrate qualities in numerous studies (Jastrow et al., 2007; Zhao et al., 2023), organic matter decomposition can be an effective index of forest soil quality changes associated with soil amendment additions.

The decomposition of standardized trembling aspen (*Populus tremuloides* Michx.) and loblolly pine (*Pinus taeda* L.) wood stakes has been proven to be an effective measure to maintain the substrate quality (e.g., nitrogen (N), C, lignin, and C:N, etc.) constant and isolate the decomposition rates as a function of soil quality changes caused by forest management (Jurgensen et al., 2006; Risch et al., 2022). For example, using the short-term (6 months) decomposition of trembling aspen, loblolly pine, and local triploid poplar (*Populus tomentosa* Carr.) wood stakes, we recently reported that even though the wood stakes of three species were in a transitional state of decay at this early stage, biochar and/or manure amendments accelerated their degradation (Zhao et al., 2022). Together with the limited increase in soil extracellular enzyme (catalase, urease, and invertase) activities after 2 years of soil amendment treatments, Zhao et al. (2022) suggested that biochar and manure had the potential to alter long-term wood decomposition and nutrient flux. However, as soil organic amendments age, their effects on soil quality might also change (Joseph et al., 2021; Khan et al., 2023). The long-term impacts of biochar and manure on surface and belowground soil processes are necessary for increasing soil C sink, improving soil quality, and mitigating climate change using biochar and other organic additives.

Therefore, we revisited our previous study site to investigate how biochar and manure would affect wood stake decomposition (mass loss) and soil extracellular enzyme activities in the longer term. Based on previous studies, we hypothesized that: Three years after the application of soil amendments, (1) biochar would be more conducive to promoting wood decomposition than manure because of its recalcitrance in the soil; (2) both amendments would increase the movement of C from wood stakes to soil and increase N into surface and mineral stakes; and (3) soil extracellular enzyme activity would be related to the amounts of added biochar and manure.

2 | MATERIALS AND METHODS

2.1 | Site description

Our study was conducted in a newly planted, short-rotation triploid poplar plantation in Guan County, Liaocheng City, Shandong Province, China (36°31′21″ N,

115°21′37″ E). The 0–30 cm soil is alkaline (pH = 8.2) with a sandy loam texture (62% sand, 35% clay, 2.7% silt). Soil chemical properties for total C, N, P, and K are 11.0, 2.2, 1.4, and 8.3 mg g^{-1}, respectively. Additional soil property details can be found in Zhao et al. (2022), and the monthly average air temperature and precipitation see Zhao et al. (2023).

2.2 | Experimental design

We employed a replicated split-split-split plot design. Our whole plots had pig manure (M; Run Dong Fertilizer Corporation, Hebei, China) applied at three rates: 0, 3, and 9 Mg ha^{-1} (abbreviated as M0, M3, and M9). Manure had a pH of 7.9, a 24% water content, and organic matter, N, P, and K contents of 450, 15, 20, and 27 mg g^{-1}, respectively (Zhao et al., 2022). Biochar (B) was the split-plot and was applied at three rates: 0, 2.5, and 10 Mg ha^{-1} (abbreviated as B0, B2.5, and B10). It was created from maize straw and was produced using slow pyrolysis at 450–500°C for 2 h in an oxygen-limited rotary furnace (Qin Feng Zhong Cheng Biomass New Material Corporation, Nanjing, China), and the resulting biochar had a pH of 8.8, N, P, K, C, and H contents of 11, 2, 3, 470, and 28 mg g^{-1}, respectively, and a C:N and H:C of 44 and 0.06. In addition, the specific surface area, pore volume, pore size, and total ash content are 5.0 m^2 g^{-1}, 6.9×10^{-3} cm^3 g^{-1}, 5.6 nm, and 44% respectively (Zhao et al., 2022). In total, we had nine soil amendment treatments (M0B0, M0B2.5, M0B10, M3B0, M3B2.5, M3B10, M9B0, M9B2.5, and M9B10) (Figure S1). Wood stake location was the split-split plot, and wood stakes were either placed horizontally on the soil surface (hereafter "surface stakes") or vertically in the mineral soil (hereafter "mineral stakes," details below). Wood stake species was the split-split-split plot, in which we used stakes created from triploid poplar, trembling aspen, and loblolly pine, hereafter poplar, aspen, and pine (details below). Thus, we had 3 manure rates × 3 biochar rates × 2 stake locations × 3 species × 3 replications; 162 experimental units total (Figure S1).

2.3 | Amendment installation

In April 2018, we applied the desired amounts of manure and biochar amendment onto the soil surface of each plot. All treatments, including the untreated M0B0, were incorporated into 20-cm-depth soil using a rotary tiller (1GQN-200, Weifang Sheng Xuan Machinery Corporation, Shandong, China). Split plots were separated by an 8-m-wide buffer strip. Although not included in our study design, this area was subsequently planted with one-year-old poplar (triploid "Beilinxiongzhu1" [(*P. alba* × *P. glandulosa*) × (*P. tomentosa* × *P. bolleana*)]) seedlings adjacent to the wood stake placement. Seedlings received furrow irrigation three times in the first month after planting, but wood stakes and soil sampling occurred outside this irrigation area. No other management activities occurred during the study period.

2.4 | Wood stake installation

Wood stake installation methods are outlined in Zhao et al. (2022) and Jurgensen et al. (2006) and described briefly here. Surface (15 cm long) and mineral (20 cm long) stakes of three species were cut from 2.5 cm × 2.5 cm kiln-dried, knot-free, sapwood stakes of 40 or 50 cm lengths with the middle 10 cm kept as a control (time = 0) to determine initial wood stake properties (Table 1) and subsequent wood stake mass loss, C and N concentrations. One end of each stake inserted into the mineral soil was treated with a wood sealer to reduce moisture loss after installation, and mineral stakes were positioned with the sealed end at the soil surface. Sapwood boards used for wood stake production were more than 50 years old.

Poplar stake carbohydrate data were from Yuan et al. (2011).

2.5 | Wood stake and soil sampling

In July 2021, 3 years after stake installation, five surface and mineral wood stakes of each species were extracted (810 stakes total). Immediately in the field, all adhering

TABLE 1 Initial chemical properties of poplar, aspen, and pine stakes (Zhao et al., 2022).

Wood stake species	Carbon (C) (%)	Nitrogen (N) (mg g^{-1})	C:N	Lignin (%)	Carbohydrate (%)	Lignin:N
Poplar	46.8	1.4	354.6	20.9[b]	67.0[c]	149.4
Aspen	46.4	1.1	421.8	20.6[a]	68.7	189.5
Pine	48.6	0.8	631.7	31.1[a]	59.6	403.7

[a]Lignin and carbohydrate content of aspen and pine stakes were from Wang et al. (2018).

[b]Poplar stake lignin data were from Wang et al. (2012).

[c]Poplar stake lignin data were from Wang et al. (2012).

material on stakes was gently removed and stakes were weighed to determine moisture content, and stakes were then transported to the laboratory.

Concurrent with stake extraction, soil samples were collected with a 3.5 cm diameter × 20 cm long auger from six random locations from each manure × biochar combination treatment. The auger was cleaned between each sample to prevent mixing residual soil with the new sample. All soil samples were sieved through a 2 mm screen to remove roots and other debris and moist soil samples were retained for extracellular enzyme analyses (in total 162 soil samples = 9 combinations [3 manure × 3 biochar] × 6 samples × 3 replications).

Wood stake mass loss was calculated by comparing the dry weight of each field stake to the weight of its corresponding control section. See Jurgensen et al. (2006) and Zhao et al. (2022) for details about mass loss and moisture content measurements.

The C and N concentrations of decomposed stake samples were determined by FLASH 2000 NC Analyzer (ThermoFisher Scientific, Cambridge, UK). Wood stake C, and N loss (or gain) was calculated by subtracting the final from the initial wood C, and N content and expressed as a percent, and the C:N ratio of stake samples was calculated by the C and N concentration.

2.6 | Soil temperature and moisture

Starting in January 2019, we measured soil temperature and moisture content at the 10 cm depth within one randomly selected replicate of three soil amendment treatments (M0B0, M0B10, and M9B10) using Onset Hobo temperature loggers and moisture sensors (Onset Computer Corporation, Bourne, Massachusetts, USA). Data were collected every 2 h until July 2021 (Figure 1).

2.7 | Extracellular enzyme analyses

Activity of soil acid phosphatase, cellulase, α-glucosidase, β-glucosidase, N-acetyl-β-D-glucosaminidase, and aryl sulfatase was determined using microanalysis methods according to Guan (1986) with enzyme-specific kits (BC0145, BC0155, BC3085, BC0165, BC4005, and BC3995, respectively; Solarbio Science & Technology Co., Beijing, China). One enzyme activity unit (per gram of soil per day) was defined thusly: acid phosphatase, release of 1 nmol of phenol under wavelength 660 nm; cellulase, production of 1 mg glucose under wavelength 540 nm; α-glucosidase, β-glucosidase, N-acetyl-β-D-glucosaminidase, and aryl sulfatase, release of 1 μmol p-nitrophenol under 340, 400, 400, and 410 nm wavelength respectively.

2.8 | Statistical analyses

For the soil enzymes, two-factor linear mixed effect (LME) models were used with manure and biochar rates as independent variables and each soil enzyme as a dependent variable (Table S1).

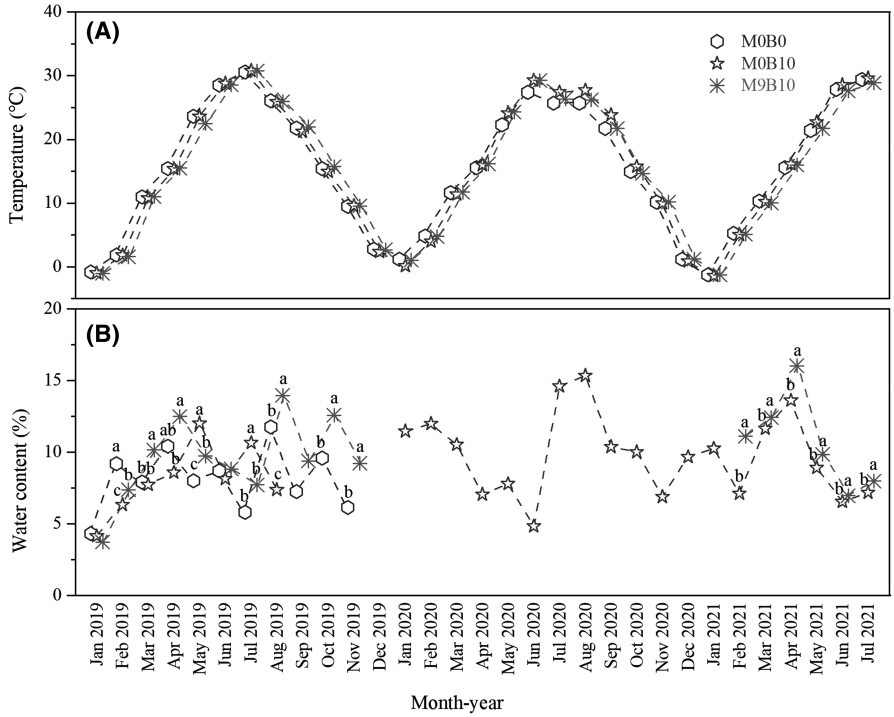


FIGURE 1 Monthly average soil temperature (A) and moisture content (B) of the non-amended M0B0, 10 Mg ha^{-1} biochar (M0B10), and 9 Mg ha^{-1} manure combined with10 Mg ha^{-1} biochar (M9B10) plot at 10 cm depth in Shandong Province, China. Different letters at the same date indicate significant differences between treatments ($p \leq 0.05$), and points with no letters are not significantly different.

For wood stakes, four-factor LME models were used with manure, biochar rates, stake location, and stake species as independent variables and each stake property (e.g., mass loss, and moisture content) as a dependent variable. Because stake location, species, and their interactions were always the main sources of variance (Table S2), post hoc Tukey's pairwise comparisons between stake species at each location were conducted. Then, data were separated and reanalyzed by stake location and species with manure and biochar rate as independent variables and wood stake properties as dependent variables (Table 2). Our goal was to investigate the potential interactions of all independent variables, and all models were a priori formulated and reported to include all second-order interaction terms.

Further, a one-way ANOVA was used to compare soil temperature and moisture content between the soil treatments with sensors (M0B0, M0B10, and M9B10). Pearson's correlation analyses were used to study the relationships between wood stake mass loss and other properties and soil enzyme activities for each species and location with all soil treatments combined.

All data were analyzed using R version 4.1.1 (R Core Team, 2021), and LME models were completed using the lmerTest package (Kuznetsova et al., 2017), in which type III tests of fixed effects were used. When the F test for a given dependent variable was significant at $p \leq 0.05$, emmeans package (Lenth et al., 2022) and Tukey–Kramer adjusted were used for post hoc comparisons. Figures were plotted by Origin Pro 2022 (OriginLab, Massachusetts, USA).

3 | RESULTS

3.1 | Soil properties

3.1.1 | Soil temperature and moisture

Soil temperature was unaffected by manure and biochar amendments (Figure 1A), but soil moisture in 2019 was significantly higher in soil treated with the high rate of biochar (M0B10) and in the combined treatment of manure and biochar (M9B10) as compared to M0B0, and this effect was observed from February to July 2021 (Figure 1B).

3.1.2 | Extracellular enzyme activities

Acid phosphatase

Three years after soil amendment treatments, soil acid phosphatase was significantly affected by biochar ($p = 0.020$), but was unaffected by manure ($p = 0.545$) and the interaction between manure and biochar ($p = 0.793$) (Table S1). Biochar applied at 2.5 Mg ha^{-1} decreased soil acid phosphatase activity as compared to no biochar application (Figure 2A).

N-acetyl-β-D-glucosidase

Manure significantly affected the production of soil N-acetyl-β-D-glucosidase ($p = 0.005$; Table S1), in which the 9 Mg ha^{-1}of manure significantly increased soil N-acetyl-β-D-glucosidase activity when compared to no manure application (Figure 2B).

α-Glucosidase

Unlike acid phosphatase, manure significantly affected soil α-glucosidase activity ($p = 0.040$) as did the interaction of manure and biochar ($p = 0.001$; Table S1). Manure applied at 9 Mg ha^{-1} increased soil α-glucosidase activity as compared to no manure application (Figure 2C). And manure and biochar interacted to increase α-glucosidase activities by 13%–37% as compared to the untreated M0B0 (Figure 2D).

Aryl sulfatase

Soil aryl sulfatase activity was significantly affected by manure ($p = 0.046$) and the manure and biochar interaction ($p = 0.004$; Table S1). Soil treated with 9 Mg ha^{-1} manure had increased aryl sulfatase activity as compared to no manure application (Figure 2E), and soil amended with manure and biochar increased aryl sulfatase activity by 10%–128% as compared to untreated M0B0 (Figure 2F).

Cellulase

Three years after soil treatments, neither biochar ($p = 0.477$), manure ($p = 0.570$), nor their combinations ($p = 0.069$) had any significant effects on cellulase activity (Table S1).

3.2 | Wood stake properties

For each variable (e.g., mass loss and moisture content), stake location and species effects were statistically significant (Table S2), which may have confounded the separation of manure and biochar treatments in the model. Therefore, as mentioned in Section 2.8, to increase the clarity of soil treatment terms, our analyses of the dependent variables were conducted separately for each wood stake location and species, and all main effects and interactions of manure and biochar were analyzed.

TABLE 2 Surface and mineral poplar, aspen, pine stake mass loss, moisture content, carbon (C) loss, nitrogen (N) gain, and C:N responses to soil manure, biochar amendments, and their interactions after 3 years of decomposition.

			Mass loss			Moisture content			C loss			N gain			C:N		
Location	**Species**	**Source of variation**	**df**	***F***	***p* values**	**df**	***F***	***p* values**	**df**	***F***	***p* values**	**df**	***F***	***p* values**	**df**	***F***	***p* values**
Surface	Poplar	Manure (M)	2	0.63	0.563	2	0.59	0.584	2	0.66	0.549	2	2.00	0.272	2	0.25	0.792
		Biochar (B)	2	11.37	**<0.001**	2	12.45	**<0.001**	2	4.69	**0.011**	2	5.21	**0.007**	2	4.25	**0.017**
		M×B	4	1.29	0.281	4	4.74	**0.002**	4	2.80	**0.030**	4	3.39	**0.012**	4	3.26	**0.015**
	Aspen	Manure (M)	2	1.17	0.403	2	1.11	0.391	2	2.46	0.203	2	2.97	0.208	2	1.65	0.312
		Biochar (B)	2	17.87	**<0.001**	2	15.22	**<0.001**	2	7.43	**0.001**	2	0.06	0.940	2	5.09	**0.008**
		M×B	4	2.73	**0.033**	4	13.54	**<0.001**	4	0.55	0.699	4	1.11	0.359	4	1.18	0.325
	Pine	Manure (M)	2	0.61	0.589	2	1.43	0.314	2	1.21	0.416	2	7.88	**0.035**	2	0.80	0.499
		Biochar (B)	2	1.65	0.198	2	5.99	**0.004**	2	0.09	0.915	2	0.13	0.882	2	0.69	0.502
		M×B	4	6.26	**<0.001**	4	4.05	**0.005**	4	2.85	**0.028**	4	1.64	0.171	4	1.18	0.325
Mineral	Poplar	Manure (M)	2	2.25	0.2053	2	6.64	0.060	2	2.61	0.079	2	0.05	0.948	2	8.71	**0.042**
		Biochar (B)	2	5.81	**0.0044**	2	3.75	**0.027**	2	**0.0044**	**0.005**	2	4.65	**0.012**	2	7.97	**<0.001**
		M×B	4	1.97	0.1068	4	7.93	**<0.001**	4	1.70	0.157	4	3.47	**0.011**	4	1.72	0.152
	Aspen	Manure (M)	2	0.55	0.5773	2	4.16	0.071	2	1.57	0.331	2	1.68	0.268	2	2.74	0.178
		Biochar (B)	2	0.90	0.4114	2	18.74	**<0.001**	2	1.39	0.253	2	0.30	0.742	2	0.44	0.647
		M×B	4	2.27	0.0670	4	9.89	**<0.001**	4	1.62	0.175	4	13.52	**<0.001**	4	5.20	**<0.001**
	Pine	Manure (M)	2	5.94	0.0591	2	15.09	**<0.001**	2	1.37	0.347	2	2.18	0.269	2	5.93	**0.042**
		Biochar (B)	2	0.90	0.4098	2	24.78	**<0.001**	2	3.17	**0.047**	2	2.48	0.089	2	6.41	**0.003**
		M×B	4	4.74	**0.0016****	4	11.01	**<0.001**	4	5.50	**<0.001**	4	21.10	**<0.001**	4	5.01	**0.001**

Note: Bold fonts denote significant effects at $p \leq 0.05$.

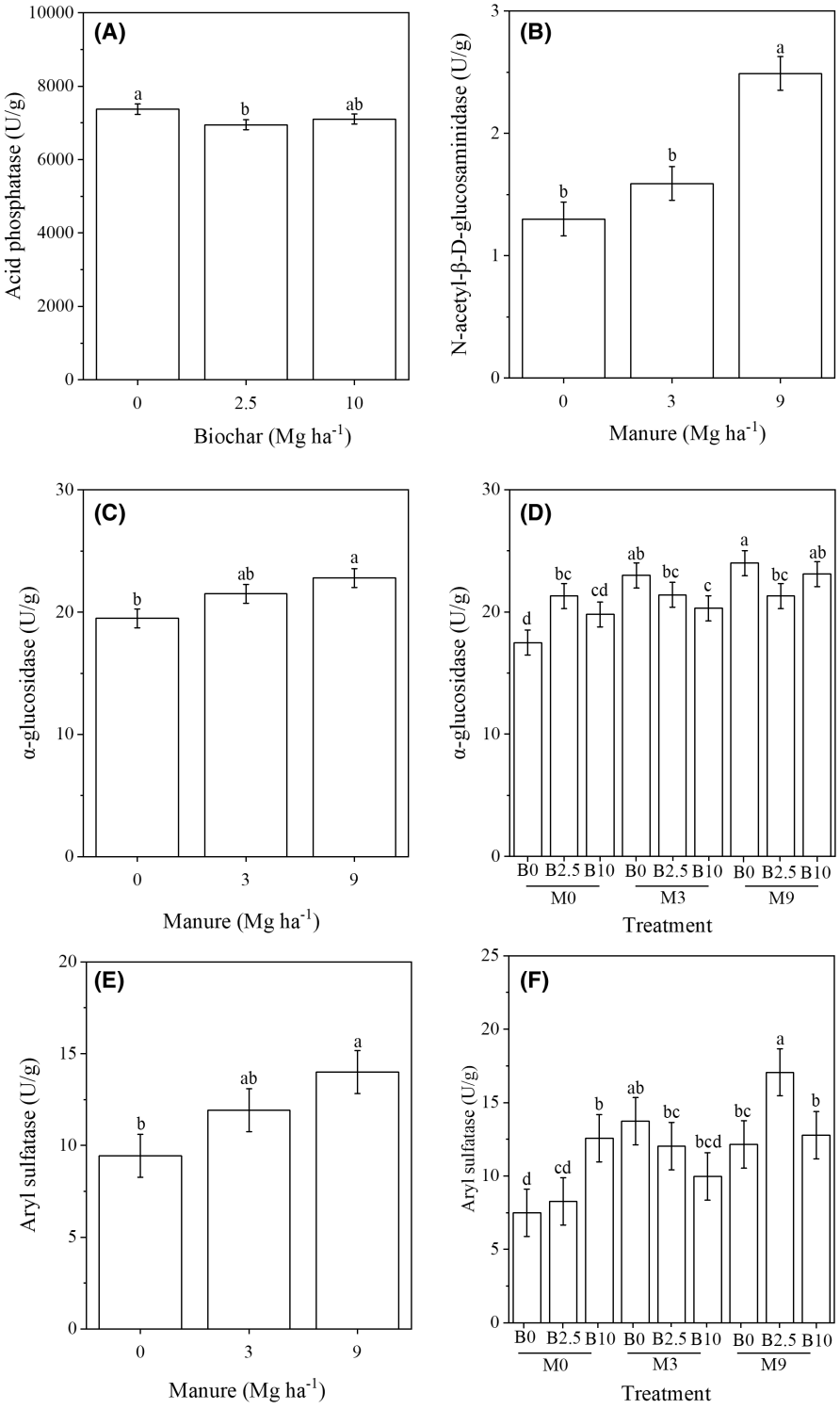


FIGURE 2 Soil acid phosphatase (A), and N-acetyl-β-D-glucosidase (B) activity as affected by the main effects of biochar and manure; soil α-glucosidase (C, D), and aryl sulfatase (E, F) activity as affected by the main effects of manure and interactions of manure and biochar. Data are means ± SE, bars with different letters indicate significantly different ($p \leq 0.05$).

3.2.1 | Soil amendment effects

Surface stakes

Poplar. Three years after wood stake installation, poplar stake moisture content, C loss, N gain, and C:N were significantly affected by manure and biochar interactions (Table 2), but the directions of change for the attributes are dissimilar. Poplar stake moisture content, C loss, and C:N were highest in the M9B10 (Figure 3A), M9B2.5 (Figure 3B), and M3B0 treatment (Figure 3D), respectively, but was not significantly different from the untreated M0B0.

As for the main effects, poplar stake properties were unaffected by manure alone (Table 2). In contrast, biochar alone significantly affected all stake variables (Table 2), in which soil treated with 2.5 and/or 10 Mg ha^{-1} biochar significantly increased wood stake mass loss, moisture content, C loss, and N gain, but decreased the C:N as compared to no biochar application (Figure S2a–e).

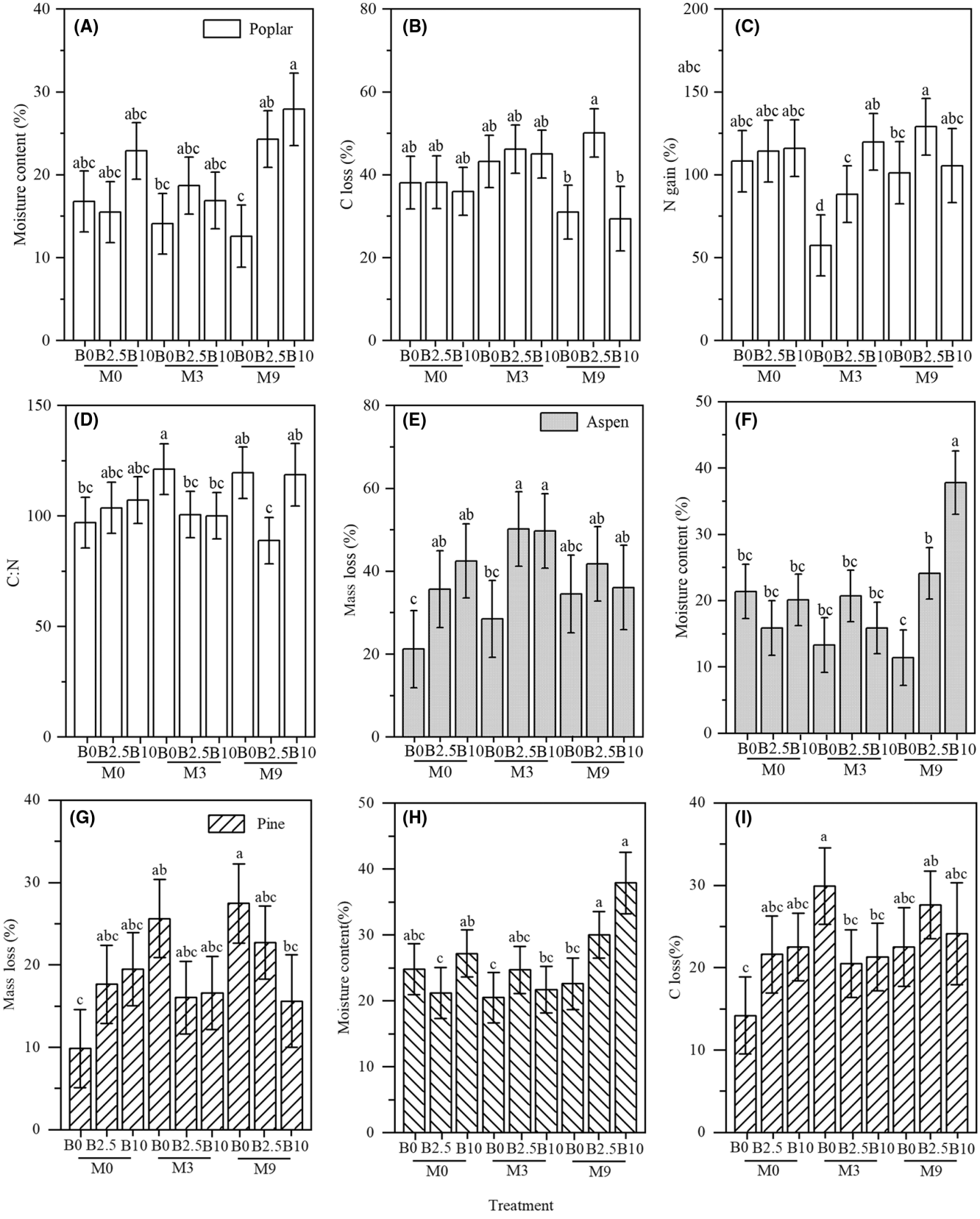


FIGURE 3 Changes in surface poplar moisture content (A), C loss (B), N gain (C), and C:N (D); Surface aspen stake mass loss (E), and moisture content (F); Surface pine stake mass loss (G), moisture content (H), and C loss (I) as affected by soil treatments after 3 years of decomposition. Data are means± SE. Different letters indicate significant differences among soil treatments ($p \leq 0.05$).

Aspen. Manure and biochar significantly interacted to affect aspen stake mass loss and moisture content (Table 2). Soil treatments (M0B2.5, M0B10, M3B2.5, M3B10, M9B2.5, and M9B10) significantly increased stake mass loss (Figure 3E), and the combination of the highest rates of manure and biochar (M9B10) increased stake moisture content as compared to stakes in M0B0 plot (Figure 3F).

Although manure alone had no significant effects on aspen stake mass loss and other properties, biochar alone did have significant effects (Table 2). Biochar at both rates caused an increased mass loss, moisture content and C loss, and lower C:N as compared to no biochar application (Figure S2f–i).

Pine. Manure and biochar interacted to significantly affect pine stake mass loss, moisture content, and C loss (Table 2). Stakes in M0B0 had significantly lower mass loss than those in M3B0 and M9B0 (Figure 3G). Pine stake moisture content was highest in the M9B10, but this was not significantly different from the untreated M0B0 (Figure 3H). More C has moved out from stakes in the M3B0 and M9B2.5 treatments than from M0B0 (Figure 3I).

Both biochar and manure alone had a significant effect on pine stake moisture content and N accumulation respectively (Table 2). Stakes in 10 Mg ha^{-1} biochar amendment had a significantly higher moisture content as compared to no biochar (Figure S2j). Further, wood stakes in 0 Mg ha^{-1} manure had more N than those in the 3 Mg ha^{-1} manure treatment, which had N levels similar to the 9 Mg ha^{-1} manure treatment (Figure S2k).

Mineral stakes

Poplar. Manure and biochar significantly interacted to affect poplar stake moisture content and N gain (Table 2). As compared to all treatments, stake moisture content was highest in the two extreme treatments, that is, M0B0 and M9B10 (Figure 4A). However, interaction effects between manure and biochar on poplar stake N gain were spurious with stakes showing gain, loss, and no changes within the treatments. But poplar stakes gained more N in the M0B2.5 and M3B10 plots as compared to the M0B0 (Figure 4B).

Biochar alone significantly affected poplar stake mass loss, moisture content, C loss, and N gain, while the C:N was affected by manure and biochar independently (Table 2). As compared to no biochar application, 10 Mg ha^{-1} biochar decreased stake mass loss and C loss (Figure S3a,c) but increased the moisture content (Figure S3b). Biochar applied at both rates increased N gains as compared to no biochar addition (Figure S3d). Independently, 2.5 Mg ha^{-1} biochar decreased, but 3 Mg ha^{-1} manure increased stake C:N as compared to their respective, unamended control plots (Figure S3e,f).

Aspen. Manure and biochar interacted to significantly affect aspen stake moisture content, N gain, and C:N

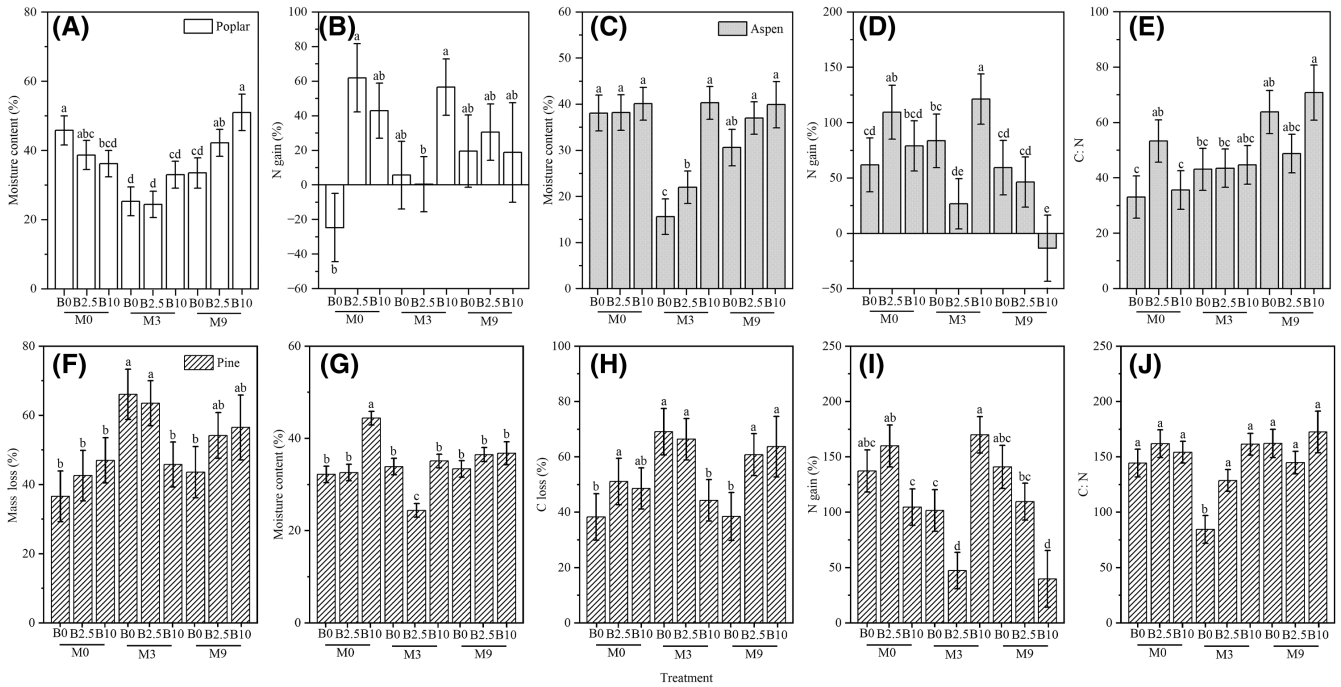


FIGURE 4 Mineral poplar stake moisture content (A), N gain (B), aspen stake moisture content (C), N gain (D), C:N (E), pine stake mass loss (F), moisture content (G), C loss (H), N gain (I), and C:N (J) as affected by soil treatments after 3 years of decomposition. Data were exhibited as means± SE. Different letters indicate significant differences among soil treatments ($p \leq 0.05$).

(Table 2). Wood stake moisture content was lowest in the M3B0 and M3B2.5 treatments as compared to M0B0 (Figure 4C). Mineral aspen stakes generally gained N except in the M9B10 treatment where stake N content was less than in the undeployed control stake (Figure 4D). Furthermore, M0B2.5 and M3B10 treatments resulted in a significantly higher N gain as compared to M0B0 (Figure 4D). The C:N of stakes in M0B2.5, M9B0, and M9B10 treatments were higher than those in the unamended M0B0 (Figure 4E).

Manure alone had no significant effects on aspen stake properties, and biochar alone was only significant for moisture content (Table 2). Aspen stakes in soil applied with 2.5 and 10 Mg ha^{-1} biochar had greater moisture content as compared to no biochar application (Figure S3g).

Pine. All pine stake properties were significantly affected by the manure and biochar interactions (Table 2). Pine stake mass loss was generally higher in the biochar treatments with and without manure, with the notable exceptions of M3B0 and M3B2.5 having the highest mass loss as compared to M0B0 (Figure 4F). Pine stake moisture content was significantly higher in M0B10 than M0B0 (Figure 4G). Changes in stake C had no clear pattern, but the C loss was less in the unamended M0B0, M3B10, and M9B0 plots as compared to other treatments (Figure 4H). Stake N was high except for the M3B2.5 and M9B10 plots (Figure 4I) and this is reflected in the C:N (Figure 4J).

Unlike aspen stakes, manure was significant for pine stake moisture content and C:N (Table 2). Biochar alone also affected stake moisture content, C loss, and C:N (Table 2). As compared to 0 Mg ha^{-1} manure and biochar, moisture content was independently decreased by 3 Mg ha^{-1} manure but increased by 2.5 Mg ha^{-1} biochar (Figure S3h,i). Moreover, 10 Mg ha^{-1} biochar increased C loss and C:N as compared to no biochar applied (Figure S3j,l).

3.2.2 | Wood stake-soil property correlations

The mass loss of stakes on the soil surface was significantly correlated with stake moisture content, C loss, C:N, and, except for poplar, N gain (Table S3). Interestingly, the positive correlation between wood stake moisture content and mass loss was only detected in surface stakes (r ranged from 0.21 to 0.54, Table S3). Further, the C loss of all surface and mineral stakes was positively (r ranged from 0.77 to 0.93) correlated with mass loss whereas C:N was negatively correlated (r ranged from −0.65 to −0.32), except mineral poplar stakes, with mass loss (Table S3). We found positive correlations with N gain (r ranged from 0.22 to 0.65) in mineral stakes and surface pine stakes ($r=0.33$), but aspen surface stakes had a negative correlation between mass loss and N gain ($r=-0.24$, Table S3).

Wood stake mass loss had little correlation with soil enzyme activity (Table S3), in which surface pine stake mass loss was significantly and positively correlated with aryl sulfatase activity ($r=0.20$, $p=0.039$), but mass loss in mineral pine stakes was significantly and negatively correlated with acid phosphatase activity ($r=-0.24$, $p=0.016$) (Table S3).

3.2.3 | Wood stake location and species effects

Wood stake location was significant for mass loss ($F=190.57$, $p<0.001$), moisture content ($F=25.36$, $p<0.001$), C loss ($F=246.34$, $p<0.001$), N gains ($F=80.73$, $p<0.001$), and C:N ($F=128.54$, $p<0.001$). As compared to surface stakes, mineral stakes had higher values for mass loss, moisture content, and C loss and lower values for N gain and C:N (Figure 5A–E).

Surface stakes

On the soil surface, the two *Populus* wood stakes had greater mass loss as compared to pine (Figure 5A), but pine stakes had the highest moisture content (Figure 5B). In addition, pine stakes had the lowest loss of C (Figure 5C) and the greatest increase in N (Figure 5D) as compared to poplar and aspen stakes. After 3 years, pine stakes had the highest C:N among all species (Figure 5E); even so, for all species, the C:N of field stakes was less than that of undeployed control stakes (Table 1).

Mineral stakes

In the mineral soil, the mass loss of the two *Populus* wood stakes was greater as compared to pine (Figure 5A). Stake moisture content was significantly greater in poplar as compared to aspen stakes, but it was similar to pine stakes and the overall moisture content difference in the three wood species was less than 5% (Figure 5B). Furthermore, pine stakes had lower C losses (Figure 5C) but higher N gains (Figure 5D) and C:N (Figure 5E) as compared to poplar and aspen stakes.

4 | DISCUSSION

While climate variables are stronger predictors of wood stake decomposition in the northern hemisphere than

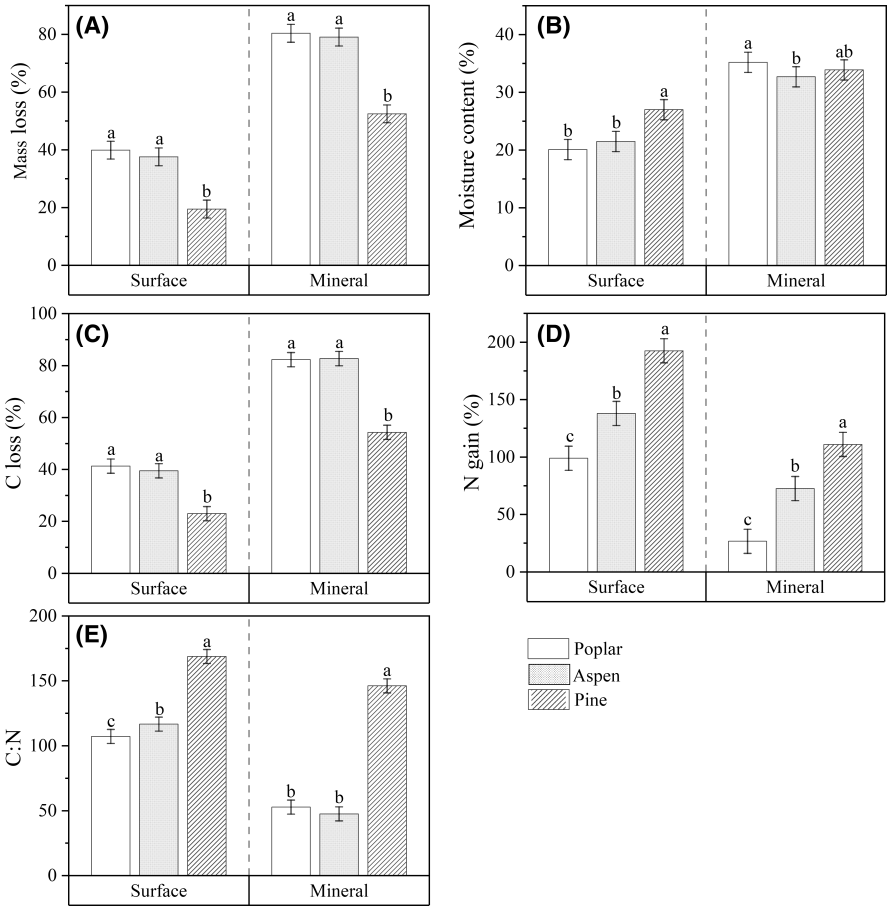


FIGURE 5 Mass loss (A), moisture content (B), carbon (c) loss (C), nitrogen (N) gains (D), and C:N (E) (means± SE) of surface and mineral stakes after 3 years of decomposition with all soil treatments combined. Different letters at the same location indicate significant differences between species ($p \leq 0.05$).

soil properties (e.g., pH and C:N), microsite climatic conditions of soil temperature and moisture also influence wood stake decay (Risch et al., 2022). In general, warmer and moister conditions facilitate the wood decay process (Monroy et al., 2022; Piaszczyk et al., 2022). Our first hypothesis was that biochar would promote wood decomposition (i.e., mass loss) more than manure because of its recalcitrance in the soil and its role in increasing soil moisture. Overall, in our study, mineral wood stakes had higher moisture content, mass loss, and C loss than surface stakes, which may be a function of the more favorable soil moisture and temperature conditions (Bradford et al., 2021; Page-Dumroese, Jurgensen, et al., 2021; Page-Dumroese, Sanchez, et al., 2021; Wang et al., 2019) compared with wood on the soil surface that is subject to greater daily fluctuations in temperature and moisture that limit fungal decay (Brooks & Kyker-Snowman, 2008; Wang et al., 2020; Zhong et al., 2017). Our results show that biochar and manure interacted to increase the moisture content of surface and mineral stakes, but biochar as an independent variable always had significant effects on wood stake moisture content, whereas manure only had a significant effect on pine stake moisture in the mineral soil (Table 2). Despite this increase in stake moisture content, mainly driven by biochar, we only observed a positive correlation between stake moisture content and mass loss for surface stakes (Table S3). Because total mass loss was greater for mineral stakes after 3 years, this suggests that soil moisture content was no longer a limiting factor for mineral wood stake decomposition. Our data show that biochar amendment increased wood stake decomposition on the soil surface mainly by an improvement in soil physical properties (i.e., moisture content) together with the changed wood stake fungal community detected in our previous study (Zhao et al., 2023) highlighted that the increased soil moisture may have driven soil microbial colonization of labile organic C (Bian et al., 2022) or the community succession (Kuramae et al., 2019) in our current study. In addition, the increased soil moisture content caused by biochar can also promote wood degradation by improving soil nutrient availability (Liu et al., 2021) and the application of mixed organic amendments (i.e., manure and biochar) to low SOM soils that are drought-prone can provide synergistic benefits (Seyedsadr et al., 2022).

Wood stake mass loss followed the order of the two *Populus* > pine, which was in line with previous research, for example, (Jurgensen et al., 2006; Page-Dumroese, Jurgensen, et al., 2021; Page-Dumroese,

Sanchez, et al., 2021; Risch et al., 2022), that wood from deciduous species, in our case poplar and aspen stakes, have less lignin, lower lignin: N, and C:N as compared to coniferous species, making them more susceptible to decay fungi (Hu et al., 2018; Perez et al., 2021). Biochar, manure, or other soil additives can affect belowground C flux directly by promoting the utilization of microbial available C, for example, original or fresh SOM; (Cui et al., 2017; Ventura et al., 2019), or indirectly by changing soil physicochemical and biological (e.g., microbial biomass and composition) properties (Steinbeiss et al., 2009; Wu et al., 2021). Generally, labile C mineralization results in the release of soluble carbohydrates and dissolved organic C into the surrounding soil (Strukelj et al., 2018), and N storage in woody substrates depends largely on the initial N concentration and C:N (Duan et al., 2018; Laiho & Prescott, 2004), in which substrates with a C:N ratio exceeding 27 ~ 30:1 may limit the growth of heterotrophic organisms (Kaye & Hart, 1997; Zimmerman et al., 1995). Thus, our second hypothesis was that manure and biochar would affect C and N flux from wood stakes, particularly C movement out and N movement in. After 3 years, manure amendments altered wood stake N concentration and C:N, but these effects varied depending on the location and species of wood stakes. Although biochar alone or combined with manure changed wood stake C, N, and C:N both on the soil surface and in the mineral soil, the nutrient release from or accumulated into the substrates was not changed. The high initial C:N of our wood stakes (>400:1) resulted in a net N gain in the stake with an order of pine > aspen > poplar (Figure 5D). In addition, we found a similar positive correlation of C loss with the mass loss for all three stake species (Table S3), as reported by Finér et al. (2016), the C moving out of wood stakes generally paralleled mass loss.

Biochar and manure can alter soil microbial community structure and function (Du et al., 2020; Xu et al., 2020), and subsequent enzyme activities (Bailey et al., 2011; Chen et al., 2018; Li et al., 2020). Biochar, characterized by high porosity and generally high C content, is known to increase soil C and water, and adsorb nutrients and other organic compounds that may promote microbial activity that leads to an increase in organic matter decomposition (Seyedsadr et al., 2022; Wardle et al., 2008). Therefore, our third hypothesis was that soil extracellular enzyme activity would be related to the amounts of added biochar and manure. Our results showed that 3 years after incorporation, manure, not biochar, increased soil enzyme activities on our coarse-textured sandy loam soil (Figure 2). The lack of a biochar effect is, however, contrary to results observed on fine-textured Andic soil in northern Idaho where biochar altered the activity of several enzymes, including N-acetyl-β-D-glucosaminidase and acid phosphatase (Shan & Coleman, 2020). This difference may be related to soil texture (coarse vs. fine) and suggests that interactions of soil texture, biochar, and manure may be important in understanding enzymatic activity.

Although manure alone had limited effects on wood stake mass loss and moisture content, and biochar alone had little effect on soil enzyme activities, they interacted to have effects on wood decomposition (Table 2) and soil enzymes (Table S1; Figure 2). Perhaps this is because the longevity and C resource of biochar can change N mineralization–immobilization–turnover of manure and change soil N composition and subsequent organic matter degradation (Ding et al., 2021). Our results were consistent with (Lentz et al., 2019; Somerville et al., 2020) that soil amendments can affect microbial activity and subsequent decomposition rates by altering soil abiotic properties, and the increased soil moisture combined with changes in other soil properties, e.g., the reduced nutrient losses when soils are sacrificed during site preparation (Gundale et al., 2016) caused by biochar amendment, may help restore many degraded soils.

Three years after the wood stake installation, we concluded that biochar, not manure, had more influence on the surface and mineral stake decomposition and moisture content, so we failed to reject our first hypothesis. Moreover, we found that biochar, not manure, had more influence on the surface and mineral stake C and N flux; thus, the weak effects of manure on C and N changes caused us to only partly accept our second hypothesis. Because biochar and manure interacted to increase soil enzyme activities, we failed to reject our third hypothesis.

5 | CONCLUSIONS

Three years after application, we found that co-application of 9 Mg ha^{-1} manure and 10 Mg ha^{-1} biochar has the potential to improve soil water retention in a sandy loam forest soil. Biochar alone, especially at 10 Mg ha^{-1}, increased above- and below-ground wood decomposition, and manure applied at 9 Mg ha^{-1} increased soil N-acetyl-β-D-glucosaminidase, α-glucosidase, and aryl sulfatase activities. Further, the synergistic benefits of biochar and manure highlight that 10 Mg ha^{-1} biochar can be used to improve degraded soil properties and mitigate climate change, particularly in short rotation forests where vegetation turnover can be rapid, and 9 Mg ha^{-1} manure can be used in combination with biochar to build SOM and provide a labile C source. Future studies are warranted to elucidate the fungal community composition changes during wood stake degradation, and the relationships

between soil properties, wood stake decomposition, and fungal communities.

AUTHOR CONTRIBUTIONS

Ruirui Zhao: Data curation; formal analysis; methodology; validation; visualization; writing – original draft. **Deborah S. Page-Dumroese:** Conceptualization; resources; writing – review and editing. **Yong Liu:** Funding acquisition; supervision. **Kai Wang:** Data curation. **R. Kasten Dumroese:** Writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available because this is a part of an on-going Ph.D. program. Data is available from the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION

The corresponding author consents on behalf of all the authors that this is original work and has permission to be published.

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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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