

## Article

# Enhancing Soil Quality of Short Rotation Forest Operations Using Biochar and Manure

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**Abstract:** Biochar and manure may be used to enhance soil quality and productivity for sustainable agriculture and forestry operations. However, the response of surface and belowground wood decomposition (i.e., soil processes) and nutrient flux to soil amendments is unknown, and more site-specific information about soil property responses is also essential. In a split-plot design, the soil was amended with three rates of manure (whole plot; 0, 3, and 9 Mg ha<sup>-1</sup>) and three rates of biochar (split-plot; 0, 2.5, and 10 Mg ha<sup>-1</sup>). Soil physical properties, nutrients, and enzyme activities were evaluated in two years. In addition, wood stakes of three species (poplar, triploid *Populus tomentosa* Carr.; aspen, *Populus tremuloides* Michx.; and pine, *Pinus taeda* L.) were installed both horizontally on the soil surface and vertically in the mineral soil to serve as an index of soil abiotic and biotic changes. Wood stake mass loss, nitrogen (N), phosphorus (P), and potassium (K) flux were tested. The high rate of both manure and biochar increased soil water content by an average of 18%, but the increase in total soil P, K, organic carbon (C) content, and enzyme activities were restricted to single sample dates or soil depths. Wood stakes decomposed faster according to stake location (mineral > surface) and species (two *Populus* > pine). On average, soil amendments significantly increased the mass loss of surface and mineral stakes by 18% and 5%, respectively, and it also altered wood stake nutrient cycling. Overall, the decomposition of standard wood stakes can be a great indicator of soil quality changes, and 10 Mg ha<sup>-1</sup> of biochar alone or combined with 9 Mg ha<sup>-1</sup> of manure can be used for long-term carbon sequestration in plantations with similar soil conditions to the present study.

**Keywords:** organic fertilizer; soil process; nutrients; enzyme activity; soil physical properties



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## 1. Introduction

Serious soil degradation problems, such as soil compaction [1] or a decrease or imbalance in soil nutrients [2] are particularly common in agricultural [3], agroforestry [4], and short-rotation forestry land [5] because of the short turnover rate of crops and intensive land use. Forests are a large and dynamic part of the global carbon (C) cycle and are valued globally for the services they provide to society [6]. In some landscapes, however, there is a trade-off between higher productivity and worsening soil degradation [7], and intensive forest operations threaten the production of fiber and biofuels [8] because of declined soil organic matter, erosion, or reduced nutrient availability. Increasing soil organic matter by using soil amendments such as composts, biochar, and other organic materials can be an important part of sustainable forestry or short-rotation forestry operations [9,10].

Industrially produced biochar is a recalcitrant C-rich material made from biomass and can be retained within the soil profile for decades to hundreds of years [11]. When used as a soil amendment, biochar can not only facilitate soil C sequestration [12] but also alleviate greenhouse effects [13]. Generally, land application of organic fertilizers, including compost, manure, or biochar, can increase soil organic matter content [14],

promote aggregate stability [15], increase nutrient retention [16], regulate soil pH [17], and increase soil cation exchange capacity and enzyme activities [18]. The combination of biochar and organic fertilizers can have gain effects. For example, manure or other organic fertilizers could make up for the lack of nutrients in biochar [19]. Additionally, biochar is a great adsorbent to reduce nutrient leaching [20], heavy metal hazards [21], as well as greenhouse gas emissions [22] from organic fertilizers. The application of one or both organic substrates can simultaneously improve site productivity while reducing soil deterioration [23].

However, biochar and/or manure amendments often produce site-specific and inconsistent soil responses in forests [24]. Whether these soil additives can improve forest soil quality and health by enhancing soil physical, chemical, and biological properties is based highly on biochar feedstock type [25], pyrolysis temperature [26], application rate or method [27], aging in soils [28], and soil texture or fertility [9]. Although soil property responses to soil amendments or other forest management operations are essential for land managers, soil quality and soil health cannot be fully measured directly [29], and more sensitive attributes or indicators are needed to assess changes in soil quality with forest land management practices.

Organic matter decomposition (both surface and belowground) is an important soil process related to energy flow and nutrient cycling in forest ecosystems [30]. The decomposition rate and nutrient flux (N, phosphorus (P), and potassium (K)) of organic matter could also comprehensively reflect soil quality (e.g., soil temperature, humidity, aggregates, nutrient availability, and microbial diversity and activity) [31], in which any soil condition changes caused by soil amendments may subsequently alter the decomposition process. Complex and inconsistent effects of soil biochar and/or manure amendments on the decomposition of organic matter exist. For instance, biochar or manure additives could lead to the mineralization or immobilization of soil original organic C [32] or accelerate the decomposition of substrate cellulose in soil [33], which results in a positive priming effect and lower net soil organic C gain [34]. Moreover, biochar and/or manure could affect soil organic matter decomposition indirectly by altering the underground biomass [35] and soil respiration [36].

In addition, soil additives or other management could also cause decomposition differences by changing substrate traits (e.g., nutrient content and lignin: N) [37,38], which makes it difficult to attribute decomposition changes to improvement in soil quality. The decomposition of standard wood stakes with heterogeneous wood properties (e.g., lignin type, lignin: cellulose ratio, and N content) [39,40] was proven to be a great measure to evaluate changes in soil condition caused by forest management operations. Using standard pine (*Pinus taeda* L.) and trembling aspen (*Populus tremuloides* Michx.) wood stakes, Adams et al. noted that inherent soil factors controlled the stake mass loss in mineral soil twenty years after clear-cut and organic matter removal in three long-term soil productivity sites in the USA [39]. Furthermore, by installing Chinese pine (*Pinus tabuliformis* Carr.), pine, and trembling aspen wood stakes in a Chinese pine forest in northern China, researchers revealed the relationship between restoration thinning measures and fungal communities [41]. Although much work has been done on the decay of wood stakes used as an index of soil changes after land management, how wood stakes may decompose and release nutrients in relation to soil biochar and manure amendments is still unclear. With increasing concern regarding the applications of biochar and manure in improving soil quality and addressing climate change [42], understanding soil properties, subsequent organic matter decomposition, and nutrient flux responses are essential, as these will provide key scientific data to relevant forest land managers and policymakers.

The current study aimed to investigate soil physical, chemical (nutrient content), and biological (enzyme activity) responses to soil manure and biochar amendments in two years. Additionally, using wood stakes of three species (native poplar, triploid *Populus tomentosa* Carr.; trembling aspen; and pine), similar to that conducted in other studies (e.g., [43–45]), we examined soil manure and biochar amendment effects on the decomposition (mass

loss), N, P, and K flux of wood stakes in a six-month decomposition process. Wood stakes were placed horizontally on the soil surface (hereafter, surface stakes) and vertically in the mineral soil (hereafter, mineral stakes), and wood stake property response was used as an index of soil quality changes. We hypothesized that soil manure and biochar amendments would: (1) increase the mass loss of both surface and mineral wood stakes; (2) alter wood stake nutrient flux; and (3) improve soil physical properties, nutrient contents, and enzyme activities.

## 2. Materials and Methods

### 2.1. Site Description

This two-year field trial was conducted in Guan County, Liaocheng City, Shandong province, China (36°31'21" N, 115°21'37" E) at an elevation of 46 m. This area experiences a semi-arid continental climate with hot, humid summers (June–August) and cold, dry winters (December–February). The average annual rainfall at the site is 550 mm, with 60% of the precipitation occurring in the summer, and the mean annual temperature and evaporation are 13.3 °C and 1709 mm, respectively. Soil (0–20 cm) in this study site had a sandy loam texture (sand: silt: clay was 62.3%: 35.0%: 2.7%).

### 2.2. Soil Amendments and Wood Stakes

Maize straw biochar was produced by Qinfeng Zhong Cheng Biomass New Material Corporation (Nanjing, China) through slow pyrolysis at 450–500 °C for 2 h in an oxygen-limited rotary furnace, and pig manure was purchased from Run Dong Fertilizer Corporation (Shijiazhuang, China). Biochar and manure characterization was carried out before the initiation of this field trial (see Section 2.5 and Table 1).

**Table 1.** Average initial chemical properties of sandy loam soil at two depths, maize straw biochar, and pig manure used in Shandong Province, China.

Property	Mineral Soil Depth		Maize Straw Biochar	Pig Manure
	0–30 cm	30–60 cm		
Total N (mg g <sup>-1</sup> )	2.2	1.6	10.7	14.6
Total P (mg g <sup>-1</sup> )	1.4	1.8	1.6	19.5
Total K (mg g <sup>-1</sup> )	8.3	7.5	2.6	26.8
pH	8.2	7.5	8.8	7.9
Organic matter content (g kg <sup>-1</sup> )	7	6	-	450
C (%)	1.1	1.0	48	-
C:N	5.0	6.1	44	-
Hydrogen content (g kg <sup>-1</sup> )	-	-	28	-
Specific surface area (m <sup>2</sup> g <sup>-1</sup> )	-	-	5.0	-
Pore volume (cm <sup>3</sup> g <sup>-1</sup> )	-	-	6.9 × 10 <sup>-3</sup>	-
Pore size (nm)	-	-	5.6	-
Total ash content (%)	-	-	44	-

-, not determined.

Two surface (15 cm) and two mineral (20 cm) stakes were cut from 2.5 cm × 2.5 cm kiln-dried, knot-free, sapwood 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 (see Section 2.5 and Table 2). To reduce moisture loss through the stakes after installation, one end of each mineral stake was treated with a wood sealer before installation [41,46].

**Table 2.** Initial properties of poplar, aspen, and pine stakes (mean  $\pm$  SE) and the results of ANOVA showing the differences among species.

Species	C (%)	N (mg g <sup>-1</sup> )	C: N	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )	C: P	N: P	Lignin (%)	Carbohydrate (%)	Lignin: N
Poplar	46.75 $\pm$ 0.54 <sup>b</sup>	1.4 $\pm$ 0.4 <sup>a</sup>	354.64 $\pm$ 97.84 <sup>c</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.59 $\pm$ 0.11 <sup>a</sup>	12182.77 $\pm$ 2504.18 <sup>a</sup>	28.81 $\pm$ 4.44 <sup>a</sup>	-	-	-
Aspen	46.40 $\pm$ 0.51 <sup>b</sup>	1.10 $\pm$ 0.10 <sup>b</sup>	421.81 $\pm$ 37.10 <sup>b</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	0.62 $\pm$ 0.13 <sup>a</sup>	7158.14 $\pm$ 1782.18 <sup>b</sup>	18.95 $\pm$ 1.24 <sup>b</sup>	20.64 $\pm$ 1.08 <sup>b</sup>	68.66 $\pm$ 3.38 <sup>a</sup>	189.50 $\pm$ 19.59 <sup>b</sup>
Pine	48.62 $\pm$ 1.05 <sup>a</sup>	0.8 $\pm$ 0.2 <sup>c</sup>	631.65 $\pm$ 110.29 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>b</sup>	0.29 $\pm$ 0.07 <sup>b</sup>	14646.21 $\pm$ 2756.08 <sup>a</sup>	27.86 $\pm$ 1.76 <sup>ab</sup>	31.09 $\pm$ 2.09 <sup>a</sup>	59.60 $\pm$ 2.64 <sup>b</sup>	403.72 $\pm$ 61.58 <sup>a</sup>
<i>p</i> values	0.0000 ***	0.0000 ***	0.0000 ***	0.0043 **	0.0042 **	0.0016 **	0.0146 *	0.0000 ***	0.0000 ***	0.0000 ***

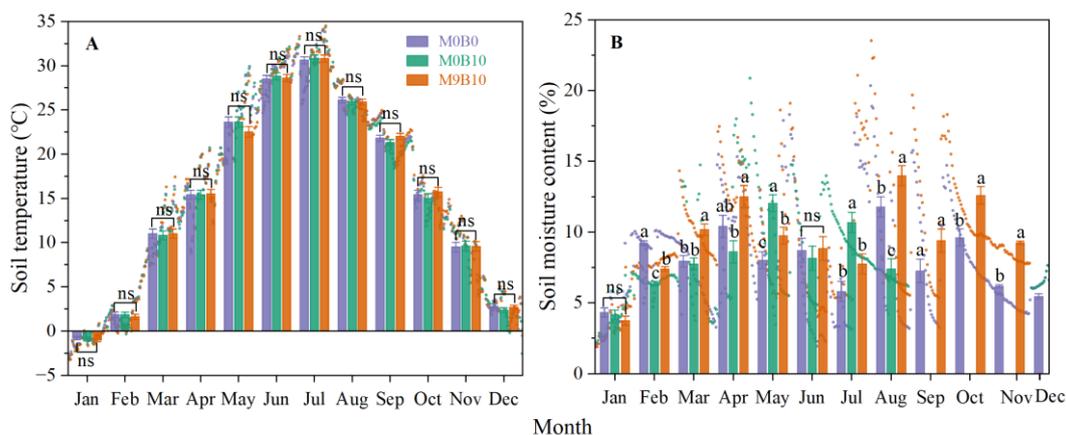
-, not determined. Different superscript lowercase letters indicate significant differences between species ( $p \leq 0.05$ ). Bold fonts with \*, \*\*, and \*\*\* indicate significant differences at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively. The lignin and carbohydrate content of aspen and pine stakes as reported by Wang et al. [46].

### 2.3. Experimental Design, Soil Treatments, and Wood Stake Installation

A split-plot experiment with three replications was applied using manure (M) at a rate of 0 (M0), 3 (M3), and 9 (M9) Mg ha<sup>-1</sup> as whole plot treatments and biochar (B) at a rate of 0 (B0), 2.5 (B2.5), and 10 (B10) Mg ha<sup>-1</sup> as split-plot treatments. Thus, the nine soil treatments were M0B0, M0B2.5, M0B10, M3B0, M3B2.5, M3B10, M9B0, M9B2.5, and M9B10, and there were 27 soil treatment plots (3 manure rates × 3 biochar rates × 3 replications). Each plot was a 24 m square with 8 m-wide strips between adjacent plots. Because soil samples were collected seasonally at two soil depths, we adopted sample date as the split-split-plot factor and soil depth as the split-split-split-plot factor for soil property analysis (see Section 2.6). For wood stakes, wood stake locations (surface and mineral) were applied as a split-split-plot factor, and three wood stake species (poplar, aspen, and pine) served as a split-split-split-plot factor. Stakes were placed in the center (2.5 m wide × 5 m long) of each soil treatment plot.

Before the trial assignment, a total of 16 mineral soil samples from two mineral soil depths (0–30 cm and 30–60 cm, 8 samples at each depth) were randomly collected from the site using a 35 mm soil auger, sieved through a 2 mm screen, and air-dried in the laboratory for chemical analysis (see Section 2.5 and Table 1). Manure and biochar were evenly applied by hand and mixed into the top 20 cm of soil using a rotary tiller (1GQN-200, Weifang Sheng Xuan Machinery Corporation, Shandong, China) in April 2018. After soil treatments, one-year-old poplar (triploid ‘Beilinxiongzhui1’ (*P. alba* × *P. glandulosa*) × (*P. tomentosa* × *P. bolleana*)) seedlings were planted with a 3 m × 4 m spacing but were not measured in this study.

In July 2018, five surface stakes of each species were placed on the soil surface and secured with a stainless-steel landscape staple. In addition, five holes (each 20 cm deep and about 30 cm apart) per wood species were created using a 2.5 × 2.5 cm coring tool, which allowed us to minimize soil compaction at the lower end of the stake. The mineral stakes were inserted into the holes with the sealed end level with the soil surface [37]. In total, 810 wood stakes were deployed in this study (nine soil treatments × three replicates × two locations (surface and mineral) × three tree species (poplar, aspen, and pine) × five individual stakes). In January 2019, Onset Hobo temperature loggers and moisture sensors (Onset Computer Corporation, Bourne, MA, USA) were embedded in the 10 cm deep soil within a single replication of the unamended M0B0, high rate of biochar (M0B10), and high rate of manure and biochar (M9B10) treatments to monitor soil temperature and water content at 2 h intervals (Figure 1).



**Figure 1.** Daily (point) and monthly (histogram, mean ± SE) average soil temperature (A) and moisture content (B) in the unamended M0B0, 10 Mg ha<sup>-1</sup> of biochar (M0B10), and high rate of both manure and biochar (M9B10) treatments at 10 cm depth in 2019. Different letters at the same date indicate significant differences between treatments ( $p \leq 0.05$ ); ns, not significant. Soil moisture data (from 10 September to 16 December) in M0B10 was lost due to instrument failure.

#### 2.4. Soil and Wood Stake Sampling

All the stakes of each species from each plot were collected and carefully extracted in January 2019 (six months after stake installation) for a total of 810 stake samples (nine soil treatments  $\times$  three replicates  $\times$  two stake locations  $\times$  three tree species  $\times$  five stakes). After removing adhering material from the surface of wood stakes, stake samples were immediately weighed in the field and then sent to the laboratory for further assessment.

Soil samples were collected during each season from April 2018 to October 2019 at three random locations within each soil treatment at two depths (0–20 cm and 20–40 cm) using a 35 mm soil auger. In each treatment and for each sample date, soil samples from each depth were composited into one sample. All soil samples were sieved through a 2 mm screen to remove roots and other debris. A subsample of moist soil was retained for extracellular enzyme analyses, and the remainder was air-dried before analysis. In April 2019, an additional 54 mineral soil cores at 0–20 cm depth (nine soil treatments  $\times$  three replicates  $\times$  two cores per plot) were collected and analyzed for soil texture. In July 2019, we randomly selected two locations within each treatment and replicate to sample soil hydraulic properties. Each undisturbed core was collected within a 100 cm<sup>3</sup> ring [47].

#### 2.5. Laboratory Assessments

Upon return from the field, stake samples were subsequently dried for 72 h at 105 °C, and a 3 cm block (subsample) was cut from the top and bottom of the mineral stake and from each end of the surface stake. All the biochar, manure, control stake, field stake subsamples, and soil samples were fine-grinded. The pH of manure, biochar, and soil was determined by a pH meter (PHM210, Radiometer Analytical, Villeurbanne Cedex, France) at a 1:5 (*w:v*) ratio. The total ash content of biochar was determined using 720 °C ignitions in a muffle furnace for 3 h. The dilution heat method was used to analyze the organic C of soil samples and manure [48,49]. Manure, biochar, control and stake subsamples, and soil were subjected to a single modified digestion method with H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> [50]. N and P concentrations were tested using a Smartchem450 Analyzer (AMS Alliance, Paris, Italy), and K concentrations were tested through SpectrAA220 (Varian, Palo Alto, CA, USA). The soil C:N ratio was calculated as the ratio of soil organic C and total N. Total C, N, and hydrogen (H) of biochar were determined by a FLASH 2000 NC Analyzer (ThermoFisher Scientific, Cambridge, UK). The BET method [51] was used to obtain the specific surface area, pore volume, and pore size of biochar (7890B Gas Chromatograph, Agilent Technologies, Santa Clara, CA, USA). Soil particle size class distribution (clay, sand, and silt content) was determined using the MasterSizer 2000 method (Malvern MasterSizer2000, Worcestershire, UK; [52]). Particles > 2 mm were considered gravel (United States Department of Agriculture (USDA) 1951). Soil electrical conductance (EC) was determined in April 2019 by a direct soil EC meter (Field Scout 2265FS, Spectrum Technologies, Inc., Chicago, IL, USA), and available N was analyzed using the alkaline hydrolysis diffusion method [53].

Four soil extracellular enzyme activities (catalase, polyphenol oxidase (PPO), urease, and invertase) were measured on the moist subsample retained from field soil sampling using a colorimetric method [54]. Briefly, urease activity was measured using 5 g of air-dried soil that was incubated at 37 °C for 24 h after the addition of a 10 mL of a 10% urea solution and 20 mL of citrate buffer (pH = 6.7). Once incubated, 3 mL of the filtrate was added to a 50 mL flask along with 4 mL of sodium phenol and 3 mL of sodium hypochlorite solutions for colorimetry at 578 nm.

For the invertase activity, 5 g of air-dried soil was incubated at 37 °C for 24 h after the addition of a 15 mL of an 8% sucrose solution and 5 mL of citrate buffer (pH = 5.5). It was then filtered, and 1 mL of filtrate was added to a 50 mL flask and treated with 3 mL of 3,5-Dinitrosalicylic acid, boiled in a water bath for 5 min, cooled for 3 min, and assessed for invertase activity using colorimetry at 508 nm.

PPO activity was measured using the spectrophotometry method, in which 1 g soil samples were mixed with 10 mL of 1% pyrogallol solution, placed into a 50 mL flask, and incubated at 30 °C for 2 h. Once incubated, 4 mL of citrate-phosphate buffer (pH = 4.5)

and 35 mL of diethyl ether were added. This mixture was placed on an orbital shaker for 30 min. PPO activity was quantified by measuring light absorbance at 430 nm.

Catalase activity was measured based on the rate of hydrogen peroxide recovery from 2 g of air-dried soil in 40 mL of distilled water that was treated with 5 mL of 0.3% hydrogen peroxide. The samples were shaken for 20 min, treated with 5 mL of 3 molL<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>, filtered, and then 25 mL of filtrate was used to titrate with 0.02 molL<sup>-1</sup> KMnO<sub>4</sub> for the presence of H<sub>2</sub>O<sub>2</sub> [54]. All determinations of enzyme activity were performed on triplicate subsamples taken from the soil composites. A calibration blank for each enzyme was run with each set of samples, and a soil-free control (an equivalent volume of deionized water instead of the soil was added) was created for urease, invertase, and PPO activity analysis. All soil enzyme colorimetry was conducted on a UV-spectrophotometer (Agilent Cary 300, San Diego, CA, USA).

## 2.6. Calculations and Statistical Analyses

Mass loss of the stakes was calculated by Equation (1):

$$\text{Mass loss (\%)} = \left( 1 - \frac{M_d}{V_d} / \frac{M_c}{V_c} \right) \times 100 \quad (1)$$

where  $M_d$  and  $V_d$  are the dry mass and volume of the samples, respectively, and  $M_c$  and  $V_c$  are the dry mass and volume of the control stakes (time = 0), respectively. Moisture content of the stakes was calculated using Equation (2):

$$\text{Moisture content (\%)} = \frac{M_f - M_d}{M_d} \times 100 \quad (2)$$

where  $M_f$  and  $M_d$  are the field mass and dry mass of the samples, respectively. N loss of stakes was calculated as using Equation (3):

$$\text{N loss (\%)} = \left( 1 - \frac{\text{sample stake N\%} \times M_d}{\text{control stake N\%} \times M_c} \right) \times 100 \quad (3)$$

where  $M_d$  and  $M_c$  are the dry mass of stake samples and control stakes, respectively. Percent P and K loss was calculated similarly.

All statistical tests were performed using R version 4.1.1 [55]. For wood stakes, four-factor linear mixed effect models (LME) were performed first using the lmerTest package [56] to examine the overall effects of manure, biochar, stake location, and stake species on the mass loss, moisture content, and N, P, and K loss of stakes. Because stake location, species, and their interactions were always the main sources of variance (Table S1), post hoc comparisons between stake species at each location were conducted. Data were separated and reanalyzed, and two-factor LME models were adopted with manure and biochar rate as independent variables and stake mass loss, moisture content, and N, P, and K loss as dependent variables.

Similarly, for soil nutrient contents and enzyme activities, four-factor LME models were performed first with manure, biochar, sample date, and soil depth as independent variables and soil nutrient content (total N, P, and K) and enzyme activities (catalase, PPO, urease, and invertase) as dependent variables. Post hoc comparisons between sample dates at each soil depth were conducted because sample date and/or soil depth and their interactions were the main sources of variance (Tables S2 and S3). Data were then separated and reanalyzed by sample date and/or soil depth. For soil physical properties derived from only one sampling date and soil depth, two-factor LME models were used directly with manure and biochar rate as independent variables and soil physical properties as dependent variables.

In all these LME models, type III tests of fixed effects were used; when the F test for a given dependent variable was significant at  $p \leq 0.05$ , we used the emmeans package [57] and Tukey–Kramer adjustment for post hoc comparisons. Furthermore, one-way ANOVA

was used to identify the initial property differences among the three wood species and the soil temperature and moisture differences among soil amendments (M0B0, M0B10 and M9B10). Pearson correlation analyses were conducted using the `rcorr` function [58,59] in the `Hmisc` package to investigate the relationships between stake properties and soil nutrients and enzyme activities for each species at each location. All figures in this study were produced using Origin Pro 2022 (OriginLab, Northampton, MA, USA).

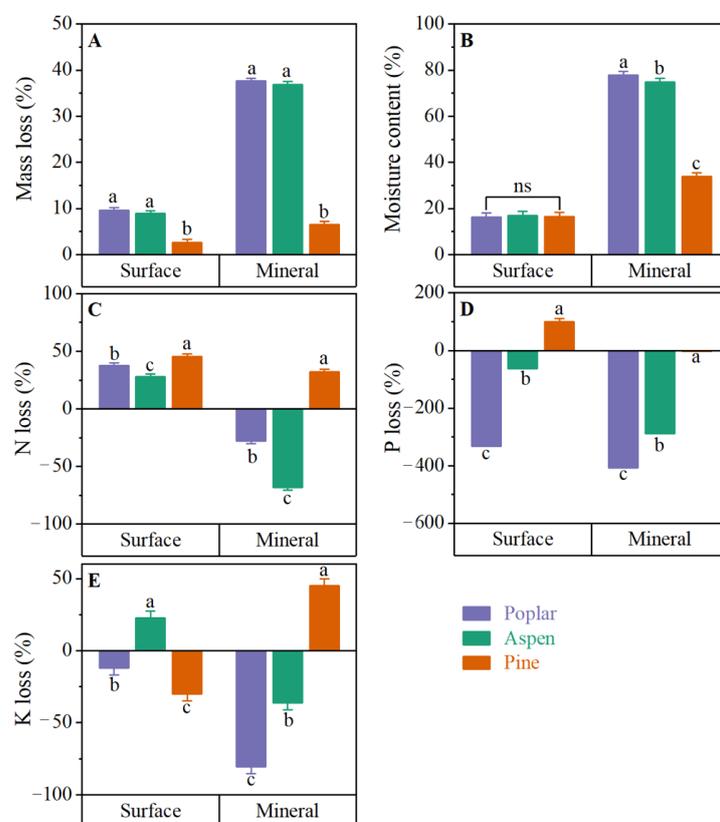
### 3. Results

#### 3.1. Wood Stake Decomposition

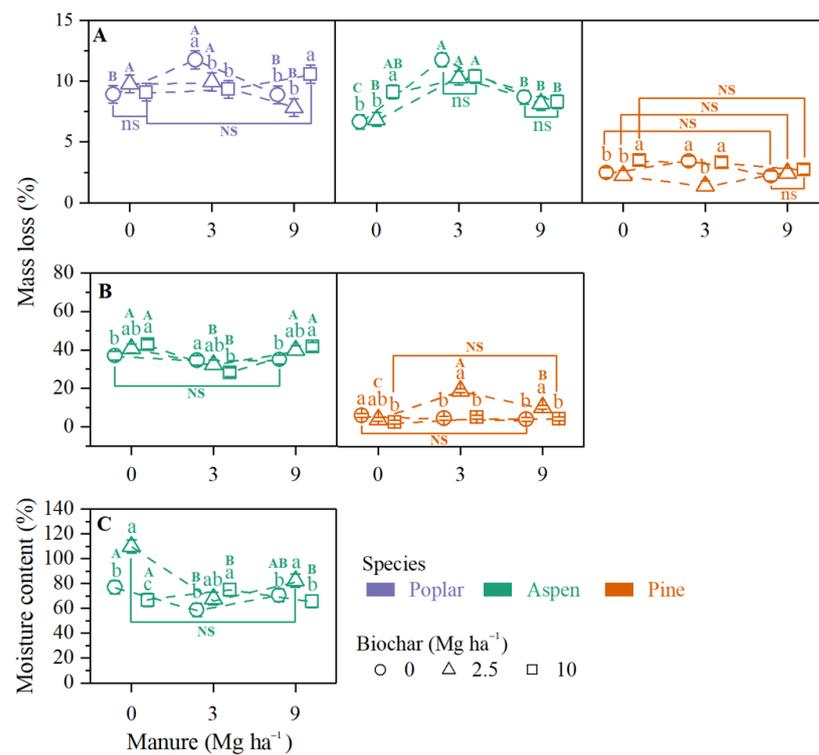
The initial chemical properties of each wood stake species were different, and it is worth noting that even the two *Populus* species often had significantly different chemical properties (Table 2).

##### 3.1.1. Wood Stake Mass Loss

Wood stakes decomposed faster in the mineral soil than on the soil surface, with notable higher mass loss found in the two *Populus* stakes as compared with pine (Figure 2A). Significant interactions among manure and biochar were reflected in the mass loss of surface stakes of three species and mineral aspen and pine stakes, while mineral poplar stake mass loss was not affected by soil amendments (Table S4). For surface stakes, the highest mass loss was detected in M3B0 (11.8%, 11.8%, and 3.4% for poplar, aspen, and pine stakes, respectively) (Figure 3). Meanwhile, when 9 Mg ha<sup>-1</sup> of manure was applied, the combination of 10 Mg ha<sup>-1</sup> of biochar (M9B10) significantly increased the mass loss of poplar stakes as compared with manure alone (M9B0), and M0B10 significantly increased the mass loss of aspen and pine stakes as compared with the unamended M0B0 (Figure 3).



**Figure 2.** The mass loss (A), moisture content (B), and nitrogen (N) (C), phosphorus (P) (D), and potassium (K) (E) loss (means  $\pm$  SE) of surface and mineral stakes with all soil treatments combined. Different letters at the same location indicate significant differences between species ( $p \leq 0.05$ ); ns, not significant.



**Figure 3.** The surface (A) and mineral (B) stake mass loss and mineral stake moisture content (C) (means  $\pm$  SE) as affected by soil amendments. Different lowercase letters indicate significant differences among biochar at each manure rate, capital letters indicate significant differences among manure at each biochar rate ( $p \leq 0.05$ ), and NS indicate no significant differences among manure at each biochar rate.

In the mineral soil, pine stake mass loss was highest in M3B2.5 (18.8%). Biochar at  $10 \text{ Mg ha}^{-1}$  (M0B10) had opposite effects on aspen and pine stake mass loss, but when  $9 \text{ Mg ha}^{-1}$  of manure was applied, the combination with biochar (M9B2.5 and M9B10) significantly increased the mass loss of aspen and pine stakes as compared with manure alone (M9B0) (Figure 3).

### 3.1.2. Wood Stake Moisture Content

The moisture content of wood stakes can reflect the soil moisture status two weeks before sampling, and to separate out the relationship of mass loss and stake moisture content we analyzed individual stake moisture contents. Not surprisingly, higher moisture content was found in the mineral stakes (average of 62.2%) as compared with stakes on the soil surface (average of 16.6%), and the moisture content in different species was in the order of poplar > aspen > pine. However, there were subtle effects ( $p > 0.05$ ) of species on the moisture of surface stakes (Figure 2B).

Significant interactions between manure and biochar were only found in the moisture content of mineral aspen, while surface aspen, surface pine, mineral poplar, and mineral pine stake moisture were only affected by biochar amendments (Table S4). As compared with no biochar addition,  $10 \text{ Mg ha}^{-1}$  of biochar significantly increased the moisture content of surface aspen and pine stakes, while the highest mineral poplar and pine stake moisture content was detected in the  $2.5 \text{ Mg ha}^{-1}$  of biochar group (Figure S1).

For mineral aspen stakes, the highest moisture content was detected in M0B2.5 (110%), and the combination of biochar and manure (M3B10 and M9B2.5) resulted in significantly higher moisture content as compared with M3B0 (Figure 3). The moisture content of two surface *Populus* stakes and mineral aspen and pine stakes were significantly correlated with the stake mass loss ( $r$  from 0.28 to 0.35) (Table 3).

**Table 3.** Pearson correlation between wood stake and soil properties with all treatments combined.

Location and Species		Wood Stake				Soil Properties									
		Moisture Content	N Loss	P Loss	K Loss	Total N	Total P	Total K	Soil Organic Carbon	Available N	C:N	Catalase	PPO	Urease	Invertase
Pearson Correlation Coefficients (r)															
Surface Poplar	Mass loss	<b>0.32 ***</b>	0.02	<b>0.28 ***</b>	0.09	0.04	−0.15	0.02	0.15	0.14	0.12	0.08	0.09	0.03	<b>0.18 *</b>
	N loss					<b>0.25 **</b>	−0.01	0.09	<b>0.20 *</b>	0.04	−0.06	<b>0.18 *</b>	−0.07	0.05	−0.07
	P loss					−0.03	−0.06	0.05	0.00	0.01	0.05	0.02	0.09	0.08	0.06
	K loss					<b>0.17 *</b>	−0.13	0.15	0.16	0.07	0.00	<b>0.17 *</b>	0.01	0.10	−0.01
Aspen	Mass loss	<b>0.28 ***</b>	<b>−0.40 ***</b>	<b>−0.41 ***</b>	<b>−0.20 *</b>		−0.08		0.04	0.06		0.08		0.14	<b>0.18 *</b>
	N loss				−0.14	0.10	<b>−0.18 *</b>	−0.16	0.02	−0.03	0.07	0.11	−0.06	0.03	
	P loss				0.09	0.11	−0.16	−0.06	0.00	<b>−0.18 *</b>	−0.08	−0.01	−0.08	0.07	
	K loss				0.15	−0.01	−0.05	0.03	−0.10	−0.13	−0.01	<b>−0.20 *</b>	−0.06	−0.09	−0.09
Pine	Mass loss	0.12	<b>−0.27 **</b>	−0.01	−0.09		0.02	0.02	0.02	0.07		−0.02	0.04	−0.05	
	N loss				0.00	0.02	−0.07	−0.01	0.03	−0.02	0.12	0.08	0.06	−0.09	
	P loss				−0.04	0.07	−0.02	0.03	<b>−0.25 **</b>	0.09	0.04	−0.02	0.05	0.15	
	K loss				<b>−0.17 *</b>	0.05	−0.10	−0.14	−0.03	0.03	−0.07	−0.07	0.05	−0.06	
Mineral Poplar	Mass loss	0.16	<b>0.30 ***</b>	<b>0.18 *</b>	0.13		0.02	−0.09		−0.10	0.05		0.13	0.02	
	N loss				0.08	−0.11	0.02	0.17	0.15	0.07	0.06	0.05	−0.05	0.06	
	P loss				0.12	0.03	0.05	0.06	−0.0	−0.08	<b>0.18 *</b>	0.04	<b>0.18 *</b>	0.10	
	K loss				0.01	−0.03	0.06	−0.05	0.01	−0.07	0.17	−0.01	0.14	0.03	
Aspen	Mass loss	<b>0.31 ***</b>	−0.16	0.13	−0.06		−0.10	0.03		−0.01		−0.01	0.03	0.09	
	N loss				−0.08	−0.07	0.07	0.02	0.01	0.12	−0.05	0.17	−0.09	0.02	
	P loss				−0.15	−0.01	−0.09	−0.05	0.01	0.11	−0.08	0.10	−0.06	0.07	
	K loss				−0.01	0.02	−0.07	0.00	−0.01	0.01	−0.11	0.13	−0.09	0.16	
Pine	Mass loss	<b>0.35 ***</b>	<b>−0.18 *</b>	<b>−0.26 **</b>	−0.16		−0.07	0.10		−0.04		−0.04	−0.04	0.04	
	N loss				−0.10	−0.11	0.05	−0.01	0.09	0.09	−0.04	−0.04	0.01	0.06	
	P loss				0.03	−0.05	−0.02	−0.10	0.12	−0.13	0.07	0.08	0.02	0.13	
	K loss				−0.09	−0.01	0.06	−0.05	−0.01	0.04	0.05	−0.04	−0.01	−0.04	

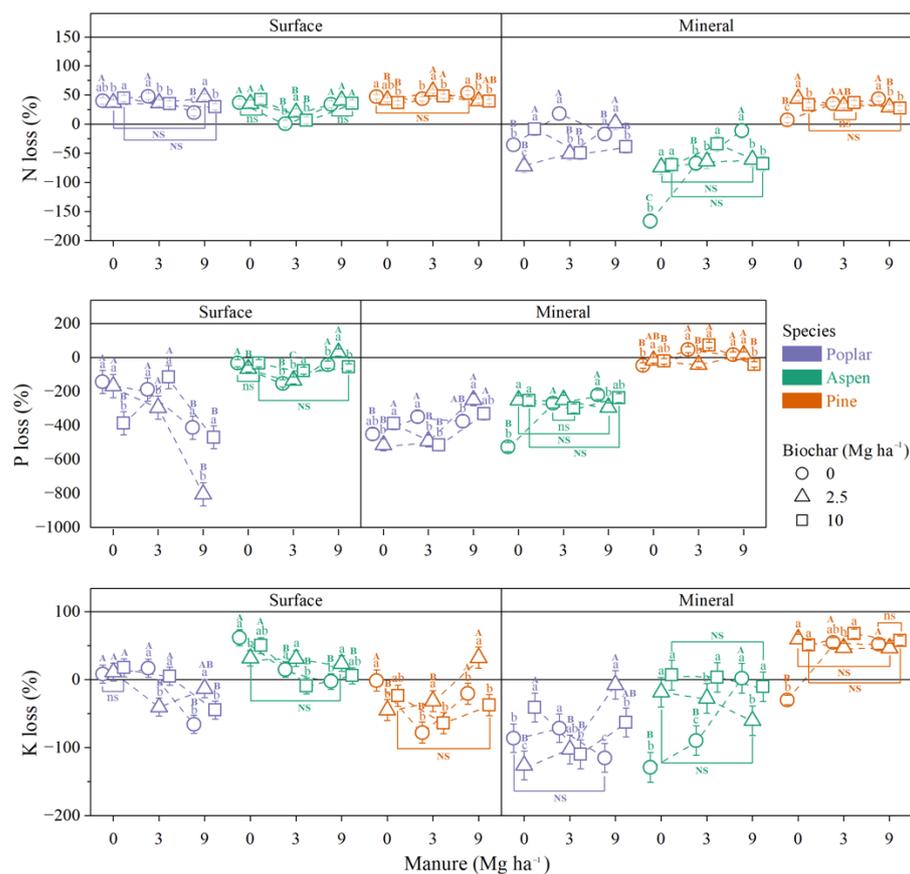
Soil properties used in this table were from all the soil samples at 0–20 cm in 2018. Bold fonts with \*, \*\*, and \*\*\* denote significant correlations at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively.

### 3.1.3. Wood Stake Nutrient Flux

Net release or accumulation of wood stake nutrients varied with the stake location and species, but there were significant effects of wood species on N, P, and K loss of both surface and mineral stakes (Figure 2C–E). Furthermore, notable interaction effects among manure and biochar were reflected in the N, P, and K loss of stakes on the soil surface and in the mineral soil, with the exception that P loss of surface pine stakes was not affected by soil amendments (Table S4).

#### Wood Stake N Loss

Although the initial pine stakes had the least amount of N as compared with the two *Populus* stakes (Table 2), they tended to have the greatest net N loss (45.2%) followed by poplar (37.4%) and aspen (28.1%) stakes (Figure 2C). N was net released from surface stakes of three species but accumulated in the two *Populus* stakes in the mineral soil (Figure 2C). For surface stakes, as manure was applied (3 or 9 Mg ha<sup>-1</sup>), the combination of biochar was more conducive to the N release from wood stakes, although exceptions to this included a higher poplar stake N loss in M3B0 and a higher pine stake N loss in M9B0. However, M0B10 significantly decreased N release from pine stakes as compared with M0B0 (Figure 4).



**Figure 4.** Nitrogen (N), phosphorus (P), and potassium (K) loss from surface and mineral stakes (means  $\pm$  SE). Different lowercase letters indicate significant differences among biochar at each manure rate, and ns indicate no significant differences; different capital letters indicate significant differences among manure at each biochar rate ( $p \leq 0.05$ ), and NS indicate no significant differences.

In the mineral soil, all soil treatments decreased the N accumulation of aspen stakes and increased the N loss of pine stakes (Figure 4). For poplar stakes, increased N accumulation was detected in M0B2.5, M3B2.5, M3B10, and M9B10, but M0B10 and M3B0 decreased N release from stakes as compared with the untreated M0B0 (Figure 4).

### Wood Stake P Loss

In general, the initial P concentration of all the wood stakes was very low (0.03–0.06 mg g<sup>-1</sup>) (Table 2). When compared with the non-deployed stakes, P was enriched in the two surface *Populus* stakes and all stakes of three species in the mineral soil, but it was released from surface pine stakes (Figure 2D). For surface stakes, significantly more P was accumulated in poplar stakes in M0B10, treatments with a high rate of manure (M9B0, M9B2.5, and M9B10), and M3B2.5 as compared with untreated M0B0 (Figure 4). As compared with manure alone (M3B0 and M9B0), the combination of biochar significantly decreased P accumulation of aspen stakes (Figure 4).

In the mineral soil, 3 Mg ha<sup>-1</sup> of manure combined with biochar (M3B2.5 and M3B10) significantly increased P accumulation in poplar stakes as compared with the M3B0 (Figure 4). When 9 Mg ha<sup>-1</sup> of manure was applied, however, the co-addition of 2.5 Mg ha<sup>-1</sup> of biochar (M9B2.5) significantly decreased P accumulation of poplar stakes as compared with M9B0. Furthermore, all soil treatments significantly decreased the P accumulation of aspen stakes, and almost all soil treatments, except M0B10, M3B2.5, and M9B10, increased P release from pine stakes as compared with M0B0 (Figure 4).

### Wood Stake K Loss

Overall, K was released from surface aspen and mineral pine stakes but accumulated in surface poplar and pine as well as two mineral *Populus* stakes (Figure 2E). On the soil surface, soil treatments with 9 Mg ha<sup>-1</sup> of manure (M9B0, M9B2.5, and M9B10) and M3B2.5 significantly increased K accumulation of poplar stakes as compared with the untreated M0B0 (Figure 4). Moreover, all soil treatments except M0B10 significantly reduced K release from aspen stakes, and all soil treatments except M9B2.5 significantly increased K accumulation of pine stakes as compared with M0B0 (Figure 4).

For mineral stakes, M0B2.5 significantly increased while M0B10 decreased K accumulation of poplar stakes as compared with M0B0. Furthermore, the combination with biochar resulted in opposite effects on poplar stake K accumulation, in which the direction was depended on the manure rate (Figure 4). However, all soil treatments decreased the K accumulation of aspen stakes and increased the K loss of pine stakes as compared with the unamended M0B0 (Figure 4). The nutrient flux and mass loss of wood stakes were closely related ( $r$  from  $-0.41$  to  $0.30$ ) (Table 3).

## 3.2. Soil Properties

### 3.2.1. Soil Physical Properties

Soil amendments, especially a high rate of both manure and biochar (M9B10), significantly increased soil moisture content as compared with the untreated M0B0 (Figure 1). Adding manure and biochar to this forest soil did not, however, generally have a measurable result on soil temperature (Figure 1) and the most measured soil physical properties, in which only five significant manure and biochar interactions in soil hydraulic properties were detected one year after soil amendments (Table S5). In the M3B0 treatment, there was a trend toward a reduction in soil maximum water holding capacity, capillary capacity, field capacity, and lower limit of optimum moisture content by 15.2%, 15.2%, 17.2%, and 14.6%, respectively, as compared with the M0B0 soil, and there was a concomitant increase in soil bulk density (Table 4).

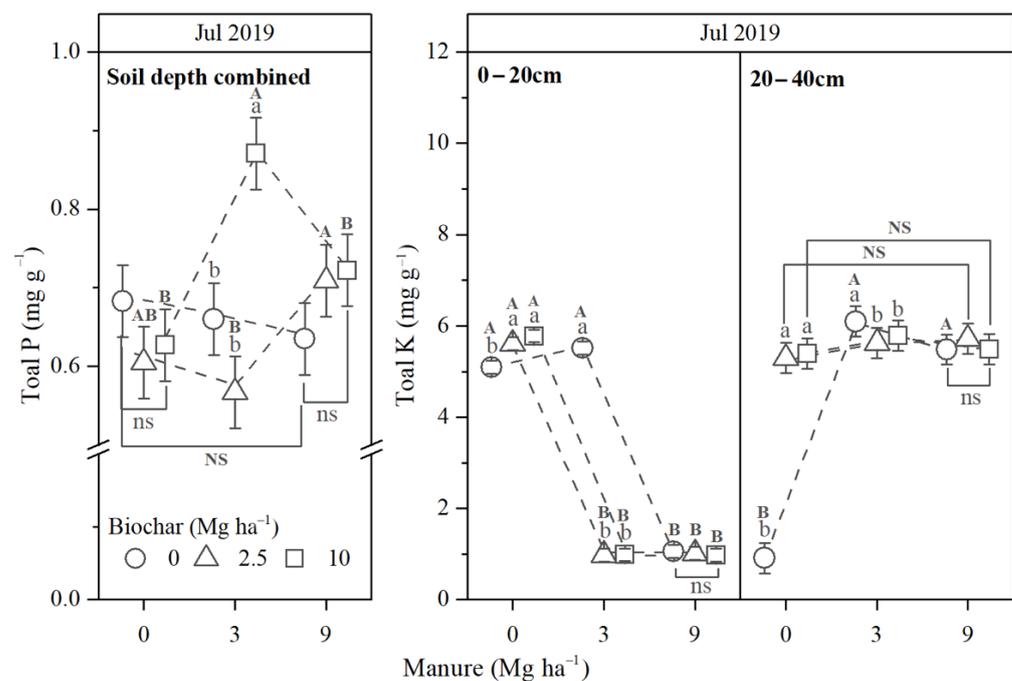
### 3.2.2. Soil Nutrients

Soil nutrients tended to be higher in 0–20 cm soil depth, and all sharply decreased in April 2019, one year after afforestation (Figure S2A–E). Manure and biochar addition had no measurable effects on soil total N, available N content, or C:N ratio (Table S2). Additionally, interactions among manure and biochar were only reflected in soil total P content in July 2019 (Table S6), in which the highest soil P (0.87 mg g<sup>-1</sup>) was detected in M3B10 (Figure 5).

**Table 4.** Changes of soil physical properties (means  $\pm$  SE) one year after manure and biochar application and incorporation into the mineral soil in Shandong Province, China.

Treatments	Soil Hydraulic Properties				
	Bulk Density (Mg m <sup>-3</sup> )	Maximum Water Holding Capacity (g kg <sup>-1</sup> )	Capillary Capacity (g kg <sup>-1</sup> )	Field Capacity (g kg <sup>-1</sup> )	Lower Limit of Optimum Moisture Content (g kg <sup>-1</sup> )
M0B0	1.20 $\pm$ 0.03 <sup>b</sup>	604 $\pm$ 29 <sup>a</sup>	585 $\pm$ 28 <sup>a</sup>	537 $\pm$ 28 <sup>ab</sup>	376 $\pm$ 19 <sup>ab</sup>
M0B2.5	1.17 $\pm$ 0.03 <sup>b</sup>	626 $\pm$ 29 <sup>a</sup>	607 $\pm$ 28 <sup>a</sup>	566 $\pm$ 28 <sup>a</sup>	396 $\pm$ 20 <sup>a</sup>
M0B10	1.19 $\pm$ 0.02 <sup>b</sup>	607 $\pm$ 22 <sup>a</sup>	590 $\pm$ 22 <sup>a</sup>	547 $\pm$ 21 <sup>a</sup>	383 $\pm$ 15 <sup>a</sup>
M3B0	1.27 $\pm$ 0.03 <sup>a</sup>	512 $\pm$ 29 <sup>b</sup>	496 $\pm$ 28 <sup>b</sup>	458 $\pm$ 28 <sup>b</sup>	321 $\pm$ 19 <sup>b</sup>
M3B2.5	1.17 $\pm$ 0.02 <sup>b</sup>	619 $\pm$ 22 <sup>a</sup>	599 $\pm$ 22 <sup>a</sup>	556 $\pm$ 21 <sup>a</sup>	389 $\pm$ 15 <sup>a</sup>
M3B10	1.17 $\pm$ 0.02 <sup>b</sup>	621 $\pm$ 22 <sup>a</sup>	604 $\pm$ 22 <sup>a</sup>	560 $\pm$ 21 <sup>a</sup>	392 $\pm$ 15 <sup>a</sup>
M9B0	1.14 $\pm$ 0.03 <sup>b</sup>	644 $\pm$ 29 <sup>a</sup>	626 $\pm$ 28 <sup>a</sup>	583 $\pm$ 28 <sup>a</sup>	408 $\pm$ 20 <sup>a</sup>
M9B2.5	1.18 $\pm$ 0.02 <sup>b</sup>	588 $\pm$ 22 <sup>a</sup>	572 $\pm$ 22 <sup>a</sup>	530 $\pm$ 21 <sup>ab</sup>	371 $\pm$ 15 <sup>ab</sup>
M9B10	1.15 $\pm$ 0.03 <sup>b</sup>	620 $\pm$ 29 <sup>a</sup>	602 $\pm$ 28 <sup>a</sup>	557 $\pm$ 28 <sup>a</sup>	390 $\pm$ 19 <sup>a</sup>

Within a column, means followed by different superscript lowercase letters indicate significant differences among treatments ( $p \leq 0.05$ ).



**Figure 5.** Soil phosphorus (P) and potassium (K) contents as affected by soil treatments (means  $\pm$  SE). Different lowercase letters indicate significant differences among biochar at each manure rate, and ns indicate no significant differences; different capital letters indicate significant differences among manure at each biochar rate ( $p \leq 0.05$ ), and NS indicate no significant differences.

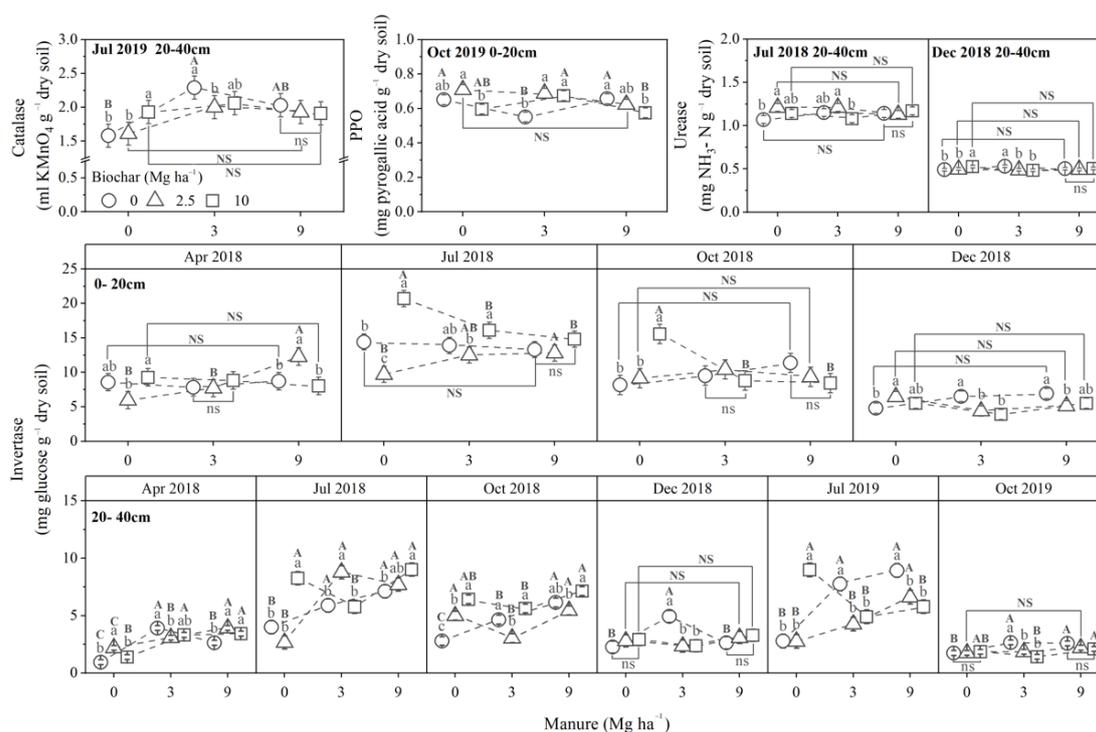
Soil total K was affected by the interactions between manure and biochar in July 2019 (Table S7), in which biochar alone (M0B2.5 and M0B10) significantly increased the K content at 0–20 cm soil depth, and all soil treatments increased the K content at 20–40 cm soil depth as compared with the unamended M0B0 (Figure 5). At 20–40 cm depth, soil K content was mainly affected by manure amendments in April 2019 (Table S7), in which 3 Mg ha<sup>-1</sup> of manure significantly increased the K content as compared with no manure addition (Figure S3A).

Soil organic C content was only affected by soil manure or biochar amendments (Table S7), in which 10 Mg ha<sup>-1</sup> of biochar as well as 3 and 9 Mg ha<sup>-1</sup> of manure could

significantly increase soil organic C content as compared with no soil amendment, but these improvements were restricted to single sample dates and soil depths (Figure S3B). Significant correlations between soil nutrients and surface stake mass or nutrient loss were detected ( $r$  from  $-0.25$  to  $0.25$ ) (Table 3).

### 3.2.3. Soil Enzyme Activities

Similar to soil nutrients, all four extracellular enzymes tested in this study tended to be higher in 0–20 cm soil depth, with the highest activities detected in July (although it sharply decreased in April 2019) one year after soil amendments and afforestation (Figure S2G–J). Significant effects of manure and biochar on soil catalase activity were only detected in Jul 2019 at 20–40 cm soil depth (Table S8), in which soil catalase was significantly higher in M0B10 and M3B0 as compared with the M0B0 (Figure 6).



**Figure 6.** Soil catalase, polyphenol oxidase (PPO), urease, and invertase activity as affected by soil treatments (means  $\pm$  SE). Different lowercase letters indicate significant differences among biochar at each manure rate, and ns indicate no significant differences; different capital letters indicate significant differences among manure at each biochar rate ( $p \leq 0.05$ ), and NS indicate no significant differences.

Soil PPO activity was mainly affected by manure amendments, and interactions between manure and biochar were only found in October 2019 at 0–20 cm soil depth (Table S8). In December 2018, soil PPO activity at 0–20 cm soil depth was significantly increased by 9 Mg ha<sup>-1</sup> of manure, but manure addition (3 or 9 Mg ha<sup>-1</sup>) tended to decrease PPO activity at 0–20 cm soil depth in October 2018 and April 2019 as compared with no manure addition (Figure S4A). In October 2019, when 3 Mg ha<sup>-1</sup> of manure was applied, the co-addition of biochar (M3B2.5 and M3B10) increased soil PPO activity as compared with manure alone (M3B0) (Figure 6).

Soil urease activity was affected by manure addition or the interactions between manure and biochar (Table S8), in which M0B2.5 significantly increased soil urease activity at 20–40 cm soil depth in July 2018 as compared with the unamended M0B0 (Figure 6). Moreover, manure application had both positive and negative effects on soil urease activity at 0–20 cm soil depth as compared with no manure addition, in which the direction was restricted to sample dates (Figure S4B).

Soil invertase activity was more sensitive to soil amendments than the other enzymes tested in this study, as significant interactions between manure and biochar were detected at almost all sample dates and soil depths (Table S8). At 0–20 cm soil depth, M0B10 and M0B2.5 significantly increased soil invertase activity in July, October, and December 2018 as compared with M0B0, while when 9 Mg ha<sup>-1</sup> of manure was applied, the co-application of 2.5 Mg ha<sup>-1</sup> of biochar (M9B2.5) significantly increased soil invertase activity as compared with manure alone in April 2018 (Figure 6).

At 20–40 cm soil depth, all soil treatments except biochar alone (M0B2.5 or M0B10) significantly increased soil invertase activity at several sample dates (from April to October 2018 and July 2019) as compared with the unamended M0B0, and soil invertase activity was highest in the M3B0 treatment in December 2018 and October 2019 (Figure 6). Soil invertase activity was positively correlated with surface poplar stake mass loss ( $r = 0.18$ ), and both positive and negative correlations among soil enzyme activities and wood stake nutrient flux were detected ( $r$  from  $-0.20$  to  $0.18$ ) (Table 3).

#### 4. Discussion

To the best of our knowledge, nothing has been reported about using the decomposition of standard organic matter to reflect soil quality changes in response to manure and biochar application in forest soils. Adding manure and biochar in this study had several positive effects on soil physical properties, nutrients, and enzyme activities in two years, and it changed the mass loss and the N, P, and K flux of wood stakes even in short-term decomposition. Standard wood stake decomposition in this study can be a great indicator of changes in soil quality or health, and wood stakes with the different properties we used also extended our understanding of soil manure and biochar effects on the soil process above the ground and under the ground in forests.

##### 4.1. Wood Stake Mass Loss and Moisture Content

Wood stakes decomposed faster in the mineral soil than on the soil surface, and the two *Populus* species decomposed faster than pine, consistent with other research [60]. The most favorable soil conditions for decomposition are associated with moderate humidity, rich in elements, well ventilated, and have neutral soil pH values. However, while the mass loss from mineral *Populus* stakes was more than 30%, pine stakes exhibited limited decomposition (2.9%), which indicated that wood properties [61], not soil conditions limited pine stake mass loss in the present study. Similar results have been found in other wood stake studies after wildfire [43] or harvesting [39,46], but this is not a universal response [40].

Consistent with our first hypothesis, soil amendments increased the mass loss of both surface and mineral stakes. This was especially true for 10 Mg ha<sup>-1</sup> of biochar alone (M0B10) or combined with 9 Mg ha<sup>-1</sup> of manure (M9B10) could hasten the native poplar stake decay on the soil surface and in the mineral soil, which can be used as a sustainable soil management practice for poplar plantations with similar soil conditions to our study. In addition, the increased wood stake moisture content on the soil surface and in the mineral soil together with data collected by moisture sensors indicated that soil manure and biochar amendments could increase soil water content in the present study, which was consistent with other research [62]. Furthermore, the positive correlations ( $r$  from 0.28 to 0.35) between moisture content and mass loss of stakes highlighted that soil moisture contributed to the faster decomposition of wood stakes. Biochar and/or manure could also improve carbon utilization efficiency and organic matter decomposition by altering the fungal: bacterial ratio and microbial structure [63].

##### 4.2. Wood Stake Nutrient Flux

Wood N, P, or K flux during decay has been shown to be a good indicator of decomposition rates in early versus later stages of decay [64]. Additionally, N content is often the main factor limiting microbial biomass growth [65], in which net N accumulation occurs in the

initial stage of wood decomposition due to its N-rich conditions (C:N ratio is ~3:1) [66]. The size and notably different initial wood properties caused by tree species and tissues could, however, also lead to various nutrient cycling patterns during wood decomposition [60,67]. Although the initial wood stake C:N ratio of three wood species is much higher than the ratio of 3:1 in our study, N was released from surface wood stakes of three species in the order of pine > poplar > aspen, which was inconsistent with a N accumulation at the first decay class of logs [67] and the external N accumulated in stumps [68]. This may relate to the dry condition on the soil surface since soil physical properties (e.g., soil moisture) could result in microbial biomass or enzyme activity changes and subsequent decomposition and nutrient flux [60].

Pine stakes that were placed in a transect from northern Finland to southern Poland showed a climatic response to N accumulation, in which stakes increased N by 17% in northern Finland, whereas in Poland, the stakes contained nearly 300% more N than the initial stakes [69]. In the present study, however, wood stake N accumulation in the mineral soil was less dramatic; this early wood stake sample date may be a transition state, and the wood may accumulate additional N [69] or release it back into the soil [70].

Nutrient addition in forest soils may change the microorganism composition and enzyme activity [71] and result in higher soil N availability [25,72], which could inhibit the N release from litter. Similarly, some of the soil amendments used in this study indeed decreased N release from surface stakes of three species. The increased N release from mineral pine stakes and N accumulation in aspen stakes may, however, relate to soil microbial changes [71,72], because soil available N content was unaffected by soil amendments and soil enzymes were minimally affected by soil amendments in our study.

In contrast to N flux, P was accumulated in surface *Populus* stakes and mineral wood stakes of three species, but it was released from surface pine stakes after six months of decomposition (Figure 2D). P content is another factor that limits the decomposition of plant material [61]; substrates with a C:P ratio > 1800 could strongly reduce microbial P mobilization [73]. However, the poor initial P content of wood stakes (the C:P ratio ranged from 7158 to 14646) in our study may stimulate decomposers (e.g., fungi and bacteria) to assimilate the required nutrients, which contributes to P accumulation. Soil manure and biochar treatments significantly increased P accumulation of surface poplar stakes but decreased the P accumulation of mineral aspen stakes, which may be because soil amendments altered the P conservation in the present nutrient-poor site by increasing soil labile carbon content [30].

K is a highly soluble nutrient during the decomposition of organic matter, and it is more likely to be leached by external environmental conditions (e.g., precipitation) and will be quickly released within a short time period after decomposition is started [74]. Logging residues were found to have most of their K released during the first year, which was attributed to increased mineralization or the high solubility of K in water [74]. The changes in K during wood decay can, however, be variable and dependent on the type of fungi within the soil profile [75]. We found that soil amendments were more conducive to K retention in surface stakes in our study, but changes were variable according to the species in the mineral soil, which could be related to altered fungal decomposers or other microbial changes.

Overall, consistent with our second hypothesis, although this early wood stake sample date may be a transition state of nutrient flux, soil additions altered wood stake nutrient flux in the six-month decomposition.

#### 4.3. Soil Physical, Chemical, and Biological Properties

Adding biochar and manure to a sandy loam soil where a short-rotation poplar forest is being grown had few effects on soil physical properties, total N, available N content, and C:N ratio in two years, but it increased soil moisture content, total P, K, organic matter content, and enzyme activities. Thus, we can partially accept our third hypothesis that soil amendments would improve soil nutrient contents and enzyme activities.

Soil organic additions are important for increasing soil moisture, soil water movement, and availability [76], especially for coarse-textured soils. However, soil hydraulic properties were unchanged by soil amendments as compared to the unamended soil in the present study, which was inconsistent with Razzaghi et al. [77], who found that biochar is more conducive to improving the hydraulic properties of coarse-textured soil as compared with those with finer textures. Soils in temperate climates, where our site is located, often show fewer responses to biochar applications than do sites in tropical or boreal climates [78]. For example, in a boreal forest with loamy sand soil, biochar applied at a rate of 5, 10, 20, or 30 Mg ha<sup>-1</sup> did not significantly increase plant-available water in the topsoil in the first year [79], but it took until the second year to see differences in bulk density, soil organic carbon, and soluble K. Similarly, soil nutrients tested in our study were barely affected by soil treatments, in which only several measurable increases in soil total P, K, and organic C content were detected, and changes were typically restricted to single sample dates or differed in soil depth. This may be related to the arid soil conditions in our study area; biochar has limited influence on soil properties in a water-limited ecosystem [80]. Biochar can also improve soil chemical properties (e.g., nutrient availability) by altering soil pH and cation exchange capacity [81]. However, soil pH was not notably affected in our study, which could be because the soil and amendment pH were already quite high. This may also be one of the main reasons why the soil nutrient contents were limitedly increased in the present study.

Soil invertase is partly responsible for the breakdown of organic matter and catalyzes the hydrolysis of sucrose, and its activity is optimized between pH 4 to 9 [82]. Soil invertase activity was increased more by soil amendments than catalase, urease, and PPO in the current field experiment, which may be related to the increased soil organic C caused by soil amendments. Soil urease activity was only notably increased in soil with 2.5 Mg ha<sup>-1</sup> of biochar (M0B2.5), but it was not as much as was detected by others [83,84], in which 5 Mg ha<sup>-1</sup> or 10 Mg ha<sup>-1</sup> of manure and/or biochar increased soil urease activity by 74.6%–96.5%. This may be due to the higher soil pH (8.2) in our present study because its activity is often limited when soil or substrate pH is greater than 6.0 [85].

Catalase and PPO are important oxidoreductases that can reduce hydrogen peroxide toxicity and catalyze the oxidation of aromatic compounds in forest soils [86]. Soil catalase activity was only significantly increased in the 3 Mg ha<sup>-1</sup> manure amendment (M3B0) in our present study. Although the addition of C-rich substrates such as manure and biochar would likely increase PPO activity [86], such results did not occur in our field trial. We assumed that the application rates of manure and biochar may have been small or that the pH was too high [87].

The results of this study indicated a limited increase in soil P, K, and organic C content, but the application of manure, biochar, or combined treatments indeed increased soil water content and enzyme activities in sandy loam soil. Furthermore, even short-term soil treatments increased the wood stake decomposition on the soil surface and in the mineral soil. In particular, treatments with 10 Mg ha<sup>-1</sup> of biochar alone or combined with 9 Mg ha<sup>-1</sup> of manure were more conducive to native poplar stake decomposition, which could be considered as a sustainable management practice in poplar plantations with sandy loam soil. However, uncertainties associated with soil applications of biochar and organic fertilizers remain, and these uncertainties call for an integrated approach to biochar and organic amendment research to maximize fossil C drawdown [88].

## 5. Conclusions

Results from the current study suggested that amending a sandy loam soil with biochar and manure, alone or in combination, could increase soil water content, but the increased soil nutrients and enzyme activities were restricted to single sample dates or differed in soil depth for the first two years. However, soil manure and biochar amendments generally increased the mass loss of surface and mineral stakes and altered the nutrient flux of three species in the short term, and results suggested that 10 Mg ha<sup>-1</sup> of biochar alone or

combined with 9 Mg ha<sup>-1</sup> of manure could be used for long-term carbon sequestration on sandy loam forests, rangelands, or agricultural areas. Field studies with standard materials can provide data on the dynamic processes that occur in and on soils and that may affect the wood over time. Additional long-term field studies about the effects of biochar and manure on wood decomposition should be considered for further soil management practices.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13122090/s1>, Table S1: The main effects and interactions of manure, biochar, stake location, and species on the wood stake mass loss, moisture content, and nitrogen (N), phosphorus (P), and potassium (K) loss after six months of decomposition; Table S2: The main effects and interactions of manure, biochar, sample date, and soil depth on the soil nutrient contents and C: N at Shandong Province, China; Table S3: The main effects and interactions of manure, biochar, sample date, and soil depth on the soil enzyme activities at Shandong Province, China; Table S4: Surface and mineral poplar, aspen, pine stake mass loss, moisture content, nitrogen (N), phosphorus (P), and potassium (K) loss responses to soil manure, biochar amendments and their interactions after six months of decomposition; Table S5: The main effects and interactions of manure and biochar amendments on the soil pH, EC and hydraulic properties at Shandong Province, China; Table S6: Soil total phosphorus (P) content responses to soil manure, biochar amendments and their interactions at each sample date with soil depth combined at Shandong Province, China; Table S7: Soil total potassium (K), and organic matter content responses to soil manure, biochar amendments and their interactions at each sample date and soil depth at Shandong Province, China; Table S8: Soil enzyme activity responses to soil manure, biochar amendments and their interactions at each sample date and soil depth at Shandong Province, China; Figure S1: Wood stake moisture content (means ± SE) as affected by the main effects of biochar amendments; Figure S2: Soil nutrient content (A–F) and enzyme activity (G–J) dynamics (means ± SE) in both 0–20 cm and 20–40 cm soil; Figure S3: Soil total potassium (K) (A) and organic carbon content (B) (means ± SE) as affected by the main effects of manure or biochar amendments; Figure S4: Soil polyphenol oxidase (PPO) (A) and urease activity (B) (means ± SE) at 0–20 cm soil as affected by the main effects of manure.

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