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Effects of Different Chinese Hickory Husk Returning Modes on Soil Nutrition and Microbial Community in Acid Forest Soil

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ABSTRACT

Chinese hickory (*Carya cathayensis* Sarg.) is an important economic forest in Southeastern China. A large amount of hickory husk waste is generated every year but with a low proportion of returning. Meanwhile, intensive management has resulted in soil degradation of Chinese hickory plantations. This study aims to investigate the effects of three Chinese hickory husk returning modes on soil amendment, including soil acidity, soil nutrition, and microbial community. The field experiment carried out four treatments: control (CK), hickory husk mulching (HM), hickory husk biochar (BC), and hickory husk organic fertilizer (OF). The phospholipid fatty acid (PLFA) biomarker method was employed to determine the soil microbial community. After one year of treatment, the results showed that: (i) HM and BC significantly increased soil pH by 0.33 and 1.71 units, respectively; (ii) HM, BC and OF treatments significantly increased the soil organic carbon, alkaline nitrogen, available phosphorous, and available potassium. The OF treatment demonstrated the most significant improvement in the soil nutrient; (iii) The soil microbial biomass significantly increased in the HM, BC and OF treatments, and all microbial groups showed an increasing trend. HM treatment increased the fungal/bacterial ratio (F/B). The OF treatment significantly decreased the Shannon-Wiener diversity (*H'*) and evenness index (*J*) of the microbial community (*P* < 0.05). Considering the treatments effects, costs, and ease of operation, our recommended returning modes of Chinese hickory husk are mulching and organic fertilizer produced by composting with manure.

KEYWORDS

Chinese hickory husk; organic fertilizer; biochar; mulching; phospholipid fatty acids; soil microbial community

1 Introduction

Chinese hickory (*Carya cathayensis* Sarg.) is an important economic forest and nut tree widely grown in Southeastern China [1,2]. Chinese hickory is well-liked by consumers for its unique taste and nutritional value. Currently, the Chinese hickory planting area is more than 86,000 ha, with an annual production of 40,000 t [3]. However, to increase production and maximize economic benefits, a large amount of chemical fertilizer, pesticide, and herbicide is applied annually. Long-term intensive management has caused soil degradation including soil erosion, soil acidification, nutrient imbalance, and reduction in soil microbial activities and diversity in Chinese hickory forests [4–7]. These problems can lead to a decline

in the quality and yield of Chinese hickory plants [8], thereby causing adverse impacts on economic and ecological benefits.

Agroforestry residues returning to fields have been widely recognized [9]. The rational utilization of wastes is beneficial to proven to increase soil fertility and have positive effects on soil microbial communities. Organic mulching can suppress weeds, reduces soil temperature fluctuations, reduce erosion, and increases total soil porosity and soil moisture [10–12], but mulches composed of organic materials offer an additional range of potential benefits including enhanced soil nutrients and improved soil microbial diversity [13,14]. Quan et al. [15] found that straw return was effective in reducing the loss of soil organic carbon. Zhou et al. [16] found that bacteria were the main driving force of straw degradation, and mulching straw increased soil bacterial biomass; Soil amendment with biochar can adjust soil acidity [17,18], and its adsorption capacity property is conducive to reducing nutrient loss, improving soil water holding capacity, and prevent leaching of plant nutrients [19,20], which also provides a favorable living environment for microorganisms to survive, reproduce and inhabit [21–23]; Organic fertilizers, such as compost and manure, are biodegradable and environmentally friendly, which makes better nutrient sources to increase crop yields. The main advantage of organic fertilizer is to increase the organic matter content of the soil. Several studies reported that the application of organic fertilizers can improve fertility and promote the formation of soil macroaggregates, in addition to increasing the water holding capacity of the soil [24,25]. Modification of soil physicochemical properties had positive effects on soil microbial biomass and activity [26].

Research based on agricultural waste resources can find a better growth environment that can provide nutrients to crops and improve yield and quality. On the other hand, it is of great importance to save resources and protect the environment. Chinese hickory husk accounts for about 60% of its total weight [27]. With the increase of hickory production, a large number of hickory husks are produced every year. The output of Chinese hickory is around 15,600 t in Lin'an City in 2020, and the husk production is about 9360 t (dry weight about 2800 t). Chinese hickory husk contains a wide variety of chemical components such as amino acids, quinones, alkaloids, and contains a large amount of nitrogen, phosphorus, and potassium [28,29]. Unique compared to other agricultural waste resources, Chinese hickory husk has a high carbon content, of which approximately 60% are lignocellulose. Lignocellulosic mulch can improve tree growth, increase soil moisture and soil carbon, as well as promote the formation and stabilization of soil aggregate [30]. The application of carbon-rich crops or organic fertilizers can increase soil fertility and also enhance the diversity of soil microorganisms [31]. Currently, Chinese hickory husks have been developed as raw materials for edible mushroom cultivation, organic fertilizer, or bioinhibitor [27,32], but a large number of hickory husks are still discarded in mountains and roadside endangering soil and water [33,34]. With the current use of hickory husks, hickory forests will lose a significant nutrient each year in the form of husks.

In this study, we selected three common modes of returning Chinese hickory husk to forests, including mulching, biochar, and organic fertilizer. The main objective of this study is to test the effects of the selected three methods on soil acidification, nutrient imbalance, and microbial community in Chinese hickory forests, resolving the contradiction between agricultural waste resources and continuous soil degradation in the forest. We hypothesized that (1) mulching and biochar can alleviate soil acidification; (2) the increase of soil C content is beneficial to improving soil microbial community structure and microbial diversity.

2 Materials and Methods

2.1 Experimental Site

The experimental sample site was located in a hickory forest in Tuankou Town (118°59'51" E, 30°4'26" N), Lin'an City, Western Zhejiang Province. The area has a subtropical monsoon climate with an average annual temperature of 18.2°C and average annual precipitation of 1448.7 mm. The altitude of the area is

340 m and the average annual daylight time is 1781.4 h [35]. Soil types are mainly yellow clay [36]. Chinese hickory tree age of this forest was 20 years and has been under intensive management for 10 years (including the long-term application of chemical fertilizers and herbicides). For fertilizer application, compound fertilizer (N:P_2O_5:K_2O, 15:15:15) with a dosage of about 350 kg hm^{-2} were used in early May and October, respectively. For the herbicide application, 20% glyphosate with a dosage of about 300 kg hm^{-2} was applied at the end of April, June, and August, respectively.

2.2 Experimental Design

The experiment was established on 14 December 2018, set up in a randomized block design with 16 plots (2 m × 2 m) in 4 blocks (20 m × 20 m each). Blocks were separated by at least 20 m. Four treatments were imposed: (1) control (CK); (2) hickory husk biochar (BC); (3) hickory husk mulching (HM); (4) hickory husk organic fertilizer (OF). Each treatment was randomly arranged in the block. The plots were located 5 m from each other and separated by a 30 cm deep sheet of 1 mm thick polyethylene film (20 cm deep belowground and 10 cm aboveground), in order to prevent the exchange of water and nutrients among the plots.

Each treatment was applied as 1.42 kg m^{-2} C. The application of hickory husk, organic fertilizer, and biochar were 2.92 kg m^{-2}, 6.13 kg m^{-2} and 2.2 kg m^{-2} (dry matter), respectively. In this experiment, for one tree, the cost of mulching was close to zero, the cost of organic fertilizer was roughly $3.7 and the cost of biochar was roughly $13. The organic fertilizer was a mixture of hickory husks and sheep manure in the ratio of 4:6 by mass, with total nitrogen of 1.36%, total phosphorus of 0.55%, total potassium of 3.03%, and total carbon of 23.28%. The hickory husks contained 0.96% total nitrogen, 0.45% total phosphorus, 3.26% total potassium, and 48.86% total carbon. The biochar was produced by anaerobic pyrolysis at 500°C for 4 h. Before application, the biochar was ground through a 2 mm sieve. The basic physicochemical properties of biochar with total carbon of 64.85%, total nitrogen of 0.44%, C/N ratio of 147.39, ash of 13.26%, and pH value of 10.02.

2.3 Soil Sampling

On 10 December 2019, soil samples were collected from the topsoil (0–20 cm) after excluding surface litter and mixed well. Soil samples were sieved through a 2 mm sieve and removed debris, fine roots, and stones. Soil samples were divided into two parts, one part was air-dried and used to determine soil organic carbon, total nitrogen, total phosphorus, and available potassium. The other part was freeze-dried and stored at −70°C for PLFA determination.

2.4 Physical and Chemical Properties of Soils

Soil physical and chemical property analysis was carried out with reference to "Soil Agrochemical Analysis" [37]. Soil pH (ratio of 1:2.5, w/v) was measured by the composite electrode method, and soil organic carbon (SOC) was determined following wet digestion with sulfuric acid (H_2SO_4) and potassium dichromate (K_2CrO_7). Alkaline nitrogen (AN) was measured by the diffusion absorption method, available phosphorous (AP) was measured by hydrochloric acid-ammonium fluoride method, and available potassium (AK) was measured by ammonium acetate extraction-flame photometer method.

2.5 Microbial Community Indices of Soils

The fatty acids in phospholipids are present only in living microbial cells and are rapidly decomposed upon the death of living cells; Therefore, PLFAs can provide direct information about the living microbial community [38]. Soil microbial community composition was determined by the phospholipid fatty acids (PLFAs) method [39]. The individual fatty acid species are indicated by nmol g^{-1} dry soil. Among each component, 14:00, 15:00, 16:00, 17:00, 18:00, i14:0, a15:0, i15:0, i15:0 3OH, 15:1ω6c, i16:0, 16:1ω7c,

a17:0, cy17:0, i17:0, i17:0 3OH, 18:1ω5c, 18:1ω7c cy19:0 indicate bacteria; 17:00, i14:0, a15:0, i15:0, i16:0, a17:0, i17:0 indicate Gram-positive bacteria; cy17:0, cy19:0, 18:1ω7c indicate Gram-negative bacteria; 10Me16:0, 10Me17:0, 10Me18:0 indicate actinomycetes; 18:1ω9c, 18:2ω6, 9c indicate fungi; 16:1ω5c indicate arbuscular mycorrhizal fungi [40,41].

The soil microbial community diversity was assessed by the Richness (SR), Shannon-Wiener index (H′), and evenness index (J) of PLFA biomarkers. These ecological parameters were calculated as follows:

Richness (SR):

$$SR = (S - 1)/In(N) \tag{1}$$

Shannon-Wiener index (H′):

$$Pi = Ni/N \tag{2}$$

$$H' = -\sum{}^{Pi} \times (InPi) \tag{3}$$

Evenness index (J):

$$J = H'/H'max \tag{4}$$

$$H'max = In(S) \tag{5}$$

where S is the number of phospholipid fatty acid species occurring in each sample, Pi is the individual as a proportion of the total, Ni is the phospholipid fatty acid content, and N is the sum of all phospholipid fatty acid contents in each sample.

2.6 Statistical Analyses

Data were analyzed using SPSS 25.0 and R 3.5.3 software. One-way analysis of variance (ANOVA) was used to compare the differences in soil physicochemical and PLFA indices between treatments ($P < 0.05$). Principal component analysis (PCA) was used to compare the changes in soil physicochemical and microbial communities between treatments. Redundancy analysis (RDA) was used to analyze the effects of changes in environmental factors on microbial communities, and both PCA and RDA were performed using the vegan package in R software.

3 Results

3.1 Physical and Chemical Properties of Soils

The results showed that hickory husk returning modes affected the physicochemical properties of the soil (Table 1). The soil pH of BC and HM treatments were significantly higher than the other two treatments ($P < 0.05$), by 1.71 and 0.33 units, respectively, but OF treatment had no significant effect on pH ($P > 0.05$). The SOC, AN, AP, and AK contents of OF treatment were the highest among the four treatments. HM, OF and BC treatments increased the soil organic carbon content ($P < 0.05$). Compared to the CK, the OF treatment had the most significant effect with an increase of 165.7%. The alkaline nitrogen content in the HM and OF treatments were higher than CK, with an increase of 22% and 62%, respectively, and the BC treatment improvement was not significant ($P > 0.05$). Compared to CK, the available phosphorous of OF treatment was 63.24 mg kg^{-1}, which significantly increased AP content by 333.74%. The BC treatment was increased by 73.04% ($P < 0.05$), and there was no significant difference between HM and CK ($P > 0.05$). The three treatments increased the available potassium content in the soil ($P < 0.05$), the enhancement effect showed that OF > BC > HM > CK, and the pairwise comparisons between these groups revealed a significant difference ($P < 0.05$).

Table 1: Effect of different treatments on soil phychemical properties

Treatments	pH	SOC (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
CK	4.72 ± 0.06 c	30.18 ± 2.11 c	125.18 ± 3.34 c	14.58 ± 0.43 c	105.88 ± 3.69 d
HM	5.05 ± 0.05 b	37.67 ± 1.43 b	153.16 ± 8.03 b	16.79 ± 0.60 c	146.9 ± 8.86 c
OF	4.7 ± 0.02 c	80.19 ± 3.52 a	203.93 ± 5.76 a	63.24 ± 3.06 a	265.1 ± 14.26 a
BC	6.43 ± 0.04 a	38.04 ± 1.66 b	136.36 ± 2.19 c	25.23 ± 0.89 b	186.6 ± 7.89 b

Note: CK was control; HM was hickory husk mulching treatment; OF was hickory husk organic fertilizer treatment; BC was hickory husk biochar treatment. SOC was soil organic carbon; AN was alkaline nitrogen; AP was Available phosphorus; AK was available potassium. The same below. Values were represented as means ± SEs (n = 4), and the different small letters within each column means significant differences at 0.05 level based on ANOVA test.

3.2 Microbial Community Structure of Soils

The total microbial biomass of BC, HM, and OF treatments were significantly higher than CK, increasing by 54.89%, 25.41%, and 22.13%, respectively ($P < 0.05$) (Fig. 1). The bacterial biomass of the four treatments were ranked as BC > OF > HM > CK, and the fungal biomass of the three treatments were higher than CK ($P > 0.05$). The BC treatment arbuscular mycorrhizal fungi (AMF) biomass was 0.80 nmol g^{-1}, which was higher than the other three treatments ($P < 0.05$), and the OF treatment of 0.21 nmol g^{-1} was the lowest among the four treatments. The BC treatment had the highest biomass of actinomycetes among the four treatments ($P < 0.05$). Three treatments had significantly higher Gram-positive bacteria and BC had the largest increase by 54.9%. Gram-positive bacteria was higher in BC and OF treatments than in CK ($P < 0.05$), and slightly increased in HM treatment ($P > 0.05$). Compared to CK, the ratio of Gram-positive bacteria/Gram-negative bacteria PLFAs (G+/G−) in OF and HM treatments were significantly different ($P < 0.05$), with a decrease of 0.32 in OF treatment. The HM treatment fungal/bacterial ratio (F/B) of 0.2 was higher than in OF ($P < 0.05$), and there was no significant difference between BC and CK ($P > 0.05$).

There was no significant difference in the richness (SR) among the four treatments (Table 2). Compared to the CK, the evenness index (J) was significantly lower in OF and BC treatments ($P < 0.05$), and there was no significant difference between HM and CK treatments ($P > 0.05$). The Shannon-Wiener diversity index (H′) in OF was the lowest among the four treatments ($P < 0.05$).

3.3 Relationships between Soil Microbial Community Changes and Environmental Factors

Combined with Pearson's correlation analysis (Fig. 2), pH was found to be positively correlated ($P < 0.01$) with soil mycorrhizal fungi, fungi, actinomycetes, Gram-positive bacteria, Gram-negative bacteria, G+/G−, and total biomass, except for F/B. The SOC, AN, and AP showed a negative correlation ($P < 0.05$) with mycorrhizal fungi and actinomycetes, and a positive correlation ($P < 0.05$) with Gram-negative bacteria. The AK was significantly and positively correlated with Gram-negative bacteria and G+/G− ($P < 0.05$).

The relationship between environmental factors and soil microbial communities was explained by redundancy analysis (RDA) (Fig. 3). The first ordination RDA axis (RDA axis 1, horizontal) explained 49.35% of the variation in the sample and the second ordination RDA axis (RDA axis 2, vertical) explained 22.15% of the total variation in the sample, both cumulatively explained 71.5% of the variation in the microbial community. On RDA 1, BC was significantly different from HM ($P < 0.05$), and HM was significantly different from OF and CK ($P < 0.05$). On RDA 2, OF was significantly different from BC ($P < 0.05$), and BC was significantly different from HM and CK ($P < 0.05$). The direction of transformation of CK compared with OF is mainly in the positive direction of RDA 2, and all the physicochemical properties are the same except for the pH value. The direction of transformation of CK

compared with HM and BC was mainly in the positive direction of RDA 1. All microbiological indicators were identical to them except for the F/B ratio.
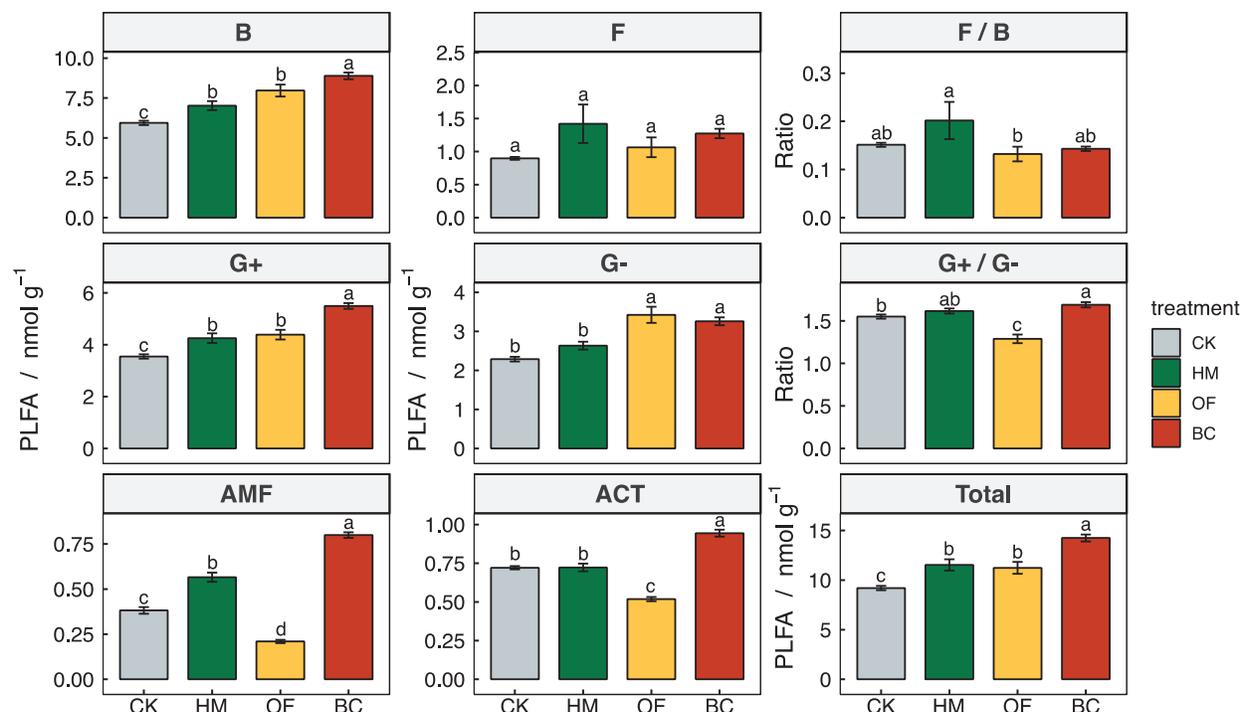

Figure 1: Comparisons of soil microbial PLFAs, and the ratios fungi/bacteria and G+/G- in different treatments

Error bars represent the standard error of the mean (n = 4). Different letters indicate significant differences at $P < 0.05$. B: bacterial PLFAs; F: fungal PLFAs; F/B: the ratio of fungal to bacterial PLFAs; G+: gram-positive bacterial PLFAs; G-: gram-negative bacterial PLFAs; G+/G−: the ratio of gram-positive bacterial to gram-negative bacterial PLFAs; AMF: arbuscular mycorrhizal fungal PLFAs; ACT: actinomycests PLFAs; Total: total PLFAs.

Table 2: Richness, diversity and Evenness indices of soil microbial communities in different treatments

Treatments	SR	J	H'
CK	3.08 ± 0.060 a	0.83 ± 0.003 a	3.12 ± 0.010 a
HM	3.07 ± 0.030 a	0.82 ± 0.002 ab	3.10 ± 0.010 a
OF	3.10 ± 0.120 a	0.79 ± 0.004 c	3.00 ± 0.020 b
BC	3.20 ± 0.060 a	0.81 ± 0.003 b	3.12 ± 0.004 a

Notes: Values were represented as means $\pm$ SEs (n = 4), and the different small letters within each column means significant difference at 0.05 level based on the ANOVA test. SR indicates richness, J indicates evenness index, and H' indicates Shannon-Wiener diversity index.

4 Discussion

4.1 Physical and Chemical Properties of Soils

Hickory husk mulching and biochar can significantly improve soil pH. BC and HM treatments contain large amounts of exchangeable cations [38] and abundant alkaloids [28,29], respectively, both of which can significantly improve soil pH. However, the hickory husks in OF treatment are decomposed after composting

and the alkaloids are degraded [42]. Moreover, various organic acids have been produced during the decomposition and transformation of organic matter in sheep manure [43], resulting in OF treatment not changing soil acidity.
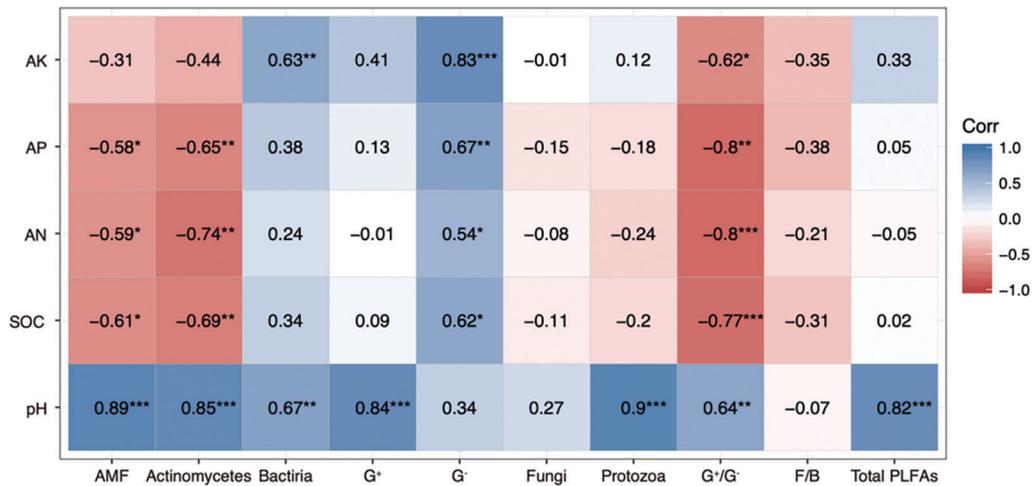

Figure 2: Correlation coefficients between physicochemical properties and soil microbial PLFAs
Values are correlation coefficients, and symbols represent the significance of the correlation coefficient. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
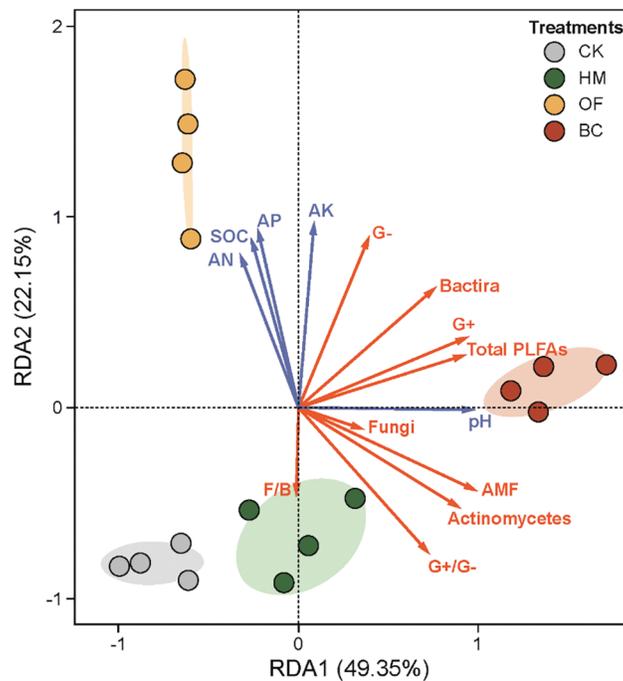

Figure 3: Redundancy analysis (RDA) of soil microbial PLFAs and physicochemical properties
Red arrows connect the soil microbial PLFAs, blue arrows connect the physical and chemical properties.

Soil organic carbon was significantly increased by different hickory husk returning modes. The OF treatment had the highest organic carbon, with an increase of 165.70% (Table 2). In OF treatment, the hickory husk and sheep manure has been decomposed into organic matter or humus [44], thus increasing the input of soil organic carbon. In BC treatment, biochar can provide a stable source of soil organic carbon due to its stable aromatic hydrocarbon structure [45], moreover, its unique microporous structure that can adsorb organic small molecules from the soil [46]. The enhancement effect of HM treatment on soil organic carbon was close to BC treatment, and the contribution of hickory husks to soil nutrients was closely related to the rate of hickory husks decomposition, which required further experiments on the rate of hickory husks decomposition [47,48]. Chinese hickory trees are mostly planted in mountainous areas. Many studies reported that increasing the soil organic carbon is conducive to reducing soil loss [49]. Mulching directly improves soil stability and soil moisture [10,11].

Compared to CK, three hickory husk returning modes can increase the content of AN, AP, and AK on the soil. In general, OF and BC treatments were more effective in enhancing nutrients than HM treatment. This may be due to the fact that hickory husk is more easily decomposed after high temperatures compost, thus more conducive to nutrient release. Han et al. [50] found that the decay and nutrient release rate of applied rotting straw was faster than the direct application of straw. Biochar, as a pyrolysis product, with a unique microporous structure and nutrient availability can lead to higher nutrient retention and nutrient availability [51]. Compared to BC and OF treatments, hickory husk mulching was slightly effective in improving soil nutrients because of the high content of hard-to-decompose substances in the hickory husk [28], which is not conducive to the release of nutrient elements from hickory husks. Significantly, hickory husk had low N and P content but high K content of about 3.25%, so the three treatments had a significant promotion of available potassium content. In addition, we also observed that the OF treatment showed the most significant increase in AN, AP and AK due to the addition of sheep manure.

4.2 Microbial Community Structure of Soils

In previous studies, other agroforestry wastes (straw, peanut shells, sheep manure, etc.) applied to soil in a similar utilization manner all significantly increased the soil microbial biomass [14,52,53]. The changes in soil pH, SOC, AN, AK, and AP by the three ways of hickory husk returning modes were closely related to the structural variation of the soil microbial community by RDA analysis (Fig. 3).

The BC treatment was the most effective in increasing soil bacterial biomass among different methods of hickory husk incorporation. This can be explained by the fact that the BC treatment contains a wide variety of organic carbon, salt-based ions and has a unique microporous structure [54,55], which improves the acidity and nutrient deficiency of hickory woodland soils and creates a soil environment conducive to bacterial growth. The arbuscular mycorrhizal fungi and actinomycetes prefer to grow in neutral or alkaline soils [56] and prefer to feed on difficult to decompose materials in the soil [57], and the biomass of both were also found to be significantly positively correlated with soil pH and negatively correlated with soil organic carbon in Pearson correlation analysis (Fig. 2). Therefore, the biomass of arbuscular mycorrhizal fungi and actinomycetes in BC and HM treatments were significantly higher than in OF treatment. In the correlation analysis, G+/G⁻ was found to be positively and significantly correlated with pH and negatively and significantly correlated with nutrient indicators (Fig. 2), so the ratio of G+/G⁻ in OF treatment was significantly decreased. Meanwhile, the G+/G⁻ ratio characterized the degree of soil nutrient stress [58], which shows that OF treatment was the most effective among the four treatments in improving the soil nutrient status.

Soil fungal biomass did not vary significantly among the different methods of hickory husks incorporation, with pairwise comparisons between these treatments were not significant ($P > 0.05$), but HM treatment fungal biomass was the highest among the four treatments (Fig. 1). The RDA analysis yielded a significant positive correlation between soil pH and fungal biomass (Fig. 3). The HM treatment

significantly improved soil pH and the fungus is the microorganism with the most ability to decompose lignin and cellulose [59], the high content of indecomposable substances in the hickory husk. Therefore, the hickory husk mulch is conducive to fungal colonization.

Both the evenness index (*J*) and the Shannon-Wiener index (*H'*) decreased significantly in OF treatment, and the evenness index (*J*) decreased significantly in BC treatment, with only HM showing no significant change. Compared to OF and BC treatments, HM treatment is similar to no-tillage and has a lower level of disturbance to the system. In addition, HM treatment has the highest F/B ratio, which also indicates that the hickory husk mulching method improves the low efficiency of resource conversion under the original intensive management and improves the system below disturbance [60,61].

5 Conclusions

In this study, hickory husk organic fertilizer has the most significant promotion effect on soil available nutrients without a decrease in pH. The hickory husk biochar and hickory husk mulching are conducive to reducing soil acidity and both the diversity and stability of soil microbial communities. It is necessary to comprehensively consider factors such as soil acidification status, material cost, and ease of operation in the management process. Our recommended returning modes of Chinese hickory husk are mulching and organic fertilizer produced by composting with manure.

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