

Article

Dynamics in Soil Microbial Communities and Soil Carbon Fractions Across Early Developmental Stages of Young Poplar Plantations (0–15 Years)

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Abstract

Soil microbial communities play a central role in terrestrial biogeochemical cycling and ecosystem functioning, and their dynamics are closely linked to soil physicochemical properties, thereby contributing to ecosystem functional stability. However, the extent to which stand age regulates soil microbial community structure and soil physicochemical properties remains insufficiently understood. In this study, black poplar (*Populus nigra*) plantations located in the hilly region of Jiangsu Province, China, were selected as a chronosequence and classified into three stand age categories: young stands (HY), middle-aged stands (HH), and late-middle-aged stands (HN). Soil samples were systematically collected from the 0–15 cm layer. Soil physicochemical properties were measured, soil DNA was extracted, and high-throughput sequencing was performed to characterize age-related changes in microbial community structure. The results showed that mineral-associated total carbon reached its highest level in middle-aged stands, whereas dissolved organic carbon, microbial biomass carbon, and soil organic carbon (SOC) were highest in late-middle-aged stands. Co-occurrence network analysis further indicated that microbial interspecific associations were most complex during the middle-aged stage. Overall, stand age induced pronounced shifts in both soil carbon fractions and microbial community organization. These findings provide new insights into the coupling relationships among stand development, soil carbon dynamics, and microbial community succession in black poplar plantations.

Keywords: poplar; stand age; soil microorganisms; soil carbon components



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1. Introduction

Poplar (*Populus* spp.) plantations are widely established in many regions of China for timber production, shelterbelts, ecological restoration, and carbon sequestration [1]. In Jiangsu Province, poplar is also planted in hilly and reclaimed areas, where early stand development can rapidly alter litter return, root activity, canopy closure, and the surface-soil microenvironment [2]. Stand age is a key factor regulating ecosystem carbon dynamics, as it influences biomass accumulation, root-derived carbon inputs, and soil carbon stability [3,4].

It is also a major determinant of soil microbial community structure and function [5]. Soil microorganisms act as a central driver of ecosystem processes, regulating nutrient cycling and organic matter decomposition, and thereby playing a fundamental role in linking plant growth with soil carbon storage [6]. Through their metabolic activities, soil microbial communities are deeply involved in carbon (C) and nitrogen (N) cycling and contribute to ecosystem functional stability [7]. During stand development, physiological traits of trees—including litter quality and root exudation patterns—change substantially. These changes, together with shifts in soil physicochemical properties, jointly reshape microbial community composition and functional potential [8]. As plantations mature, soil nutrient availability, carbon inputs, and microenvironmental conditions also undergo continuous modification, which further influences microbial ecological processes.

In recent years, the effects of stand age on soil microbial communities have become a major focus in forest ecology and soil science [9]. It has been shown that the structure and function of soil microbial communities undergo significant changes as the trees grow older [10], which are influenced by a combination of factors, including the soil physicochemical properties, plant root exudates, and types of vegetation [9]. In stand ecosystems, stands of different ages significantly influence the structure and function of the soil microbial communities through the production of different types of litter and root exudates [11]. For example, young stands typically require a greater amount of N, whereas mature stands have relatively higher demands for phosphorus (P) [12]. Moreover, the changes in soil microbial communities are also closely related to the physical and chemical properties of soil, such as the content of soil organic carbon (SOC) and N [13]. Soil microbes are highly sensitive to the dynamics of SOC across the sequences of stand age [14]. Soil microbial biomass in surface layers (0–15 cm) is particularly sensitive to SOC content and C-related stoichiometric ratios, highlighting the importance of carbon availability in regulating microbial processes [15].

However, current research on the relationship between the age of poplar plantations and soil microbes still has significant shortcomings. First, most studies have only focused on a single or limited age stage and thus lack a systematic analysis that covers the entire growth cycle from young to mature and even over-mature stands [16]. As a result, it is difficult to reveal the continuous patterns and key turning points of the microbial community dynamics as the trees age [17]. Secondly, the analysis of microbial functions mostly remains at the level of describing the community structure. The data on the composition of species obtained through high-throughput sequencing are difficult to correlate directly with their actual metabolic activities in the carbon cycle. For example, the coupling mechanisms between functional processes such as the efficiency of litter decomposition and rhizosphere carbon input transformation pathways and microbial community characteristics remain unclear. Moreover, there has only been minimal quantitative research on the interactions between the changes in plant physiological characteristics caused by tree age and the changes in soil physicochemical properties. This knowledge gap has limited a mechanistic understanding of carbon sequestration regulation in poplar plantations. Here, we investigated black poplar (*Populus nigra*) plantations widely distributed in southern Jiangsu Province, China, to clarify how stand age influences soil ecosystem characteristics. Three stand age stages were considered: young, middle-aged, and late-middle-aged stands. Specifically, this study addressed three core questions: (1) How does soil organic carbon (SOC) concentration change during stand development? (2) How does microbial community structure respond to stand age variation? (3) What are the relationships among stand development, soil physicochemical properties, and microbial communities? Accordingly, we proposed three hypotheses: (1) SOC and microbial biomass carbon contents increase with stand age [18]; (2) microbial community diversity initially decreases and subsequently increases during

stand development [19]; and (3) soil carbon fractions and nutrient availability are closely associated with microbial community composition and diversity [20].

2. Materials and Methods

2.1. Experimental Design and Sample Collection

The study site was located in the southern Jiangsu hilly region (33°05′21″ N, 119°36′55″ E) in China, which is characterized by a subtropical humid monsoon climate. The region has abundant precipitation, with an average annual temperature ranging from 15 to 17 °C, an annual mean precipitation of 1000–1200 mm, an annual mean sunshine duration of 1800–2200 h, and a frost-free period of 220–240 days. The study focused on plantations of black poplar in Liuhe, Nanjing, with different stand ages but consistent site conditions, including young stands (HY, no more than 5 years old), middle-aged stands (HH, 6–10 years old), and late-middle-aged stands (HN, 11–15 years old). But the chronosequence may include effects of site history and management. There were three replicates per plot (Table 1). Three stand ages were arranged in the order from young stand to mature stand, with a spacing of 500 m between the three replicates of each stand age. A soil auger was used to collect the soil samples within each quadrat according to the nine-grid sampling method [21]. All samples in this study were collected at approximately 50 cm from the base of the tree trunk. First, the litter was removed from the soil surface, and the soil samples were then collected from the 0–15 cm layers. The soil samples from the same plot were thoroughly mixed by layer [22]. Moreover, a ring knife was used to collect undisturbed soil to measure the soil bulk density and other indicators. The mixed soil samples were placed into labeled self-sealing bags. There was a total of nine soil samples. The nine soil samples collected were stored at a low temperature and brought back to the laboratory. After the plant debris and stones were removed, the soil was passed through a sieve. The samples were divided into two portions. One was used to determine the soil enzyme activity and other indicators, while the other was air-dried and used to determine the components of SOC and basic physicochemical properties. All the samples were stored at −80 °C before the DNA was extracted [23].

Table 1. Characteristics of Poplar Plantations Stands.

Age Cohort	Habitat Description	Geographic Coordinates	Diameter at Breast Height (DBH, cm)	Canopy Height (CH, m)	Planting Density (Plants·ha ^{−1})	Canopy Closure (CC)
young stand (HY)	Reclaimed hilly land, understory dominated by herbs.	32°26′37.9″ N, 118°48′35.8″ E	10.92 ± 0.95	9.88 ± 0.33	662.67 ± 14.19	0.70 ± 0.05
middle-aged stand (HH)	Sloping hilly land with herb-shrub understory	32°26′28.4″ N, 118°48′14.2″ E	16.98 ± 1.21	14.02 ± 0.98	443.67 ± 10.50	0.86 ± 0.08
late-middle-aged stand (HN)	Undisturbed land with herb-shrub understory	32°25′57.7″ N, 118°46′32.1″ E	23.01 ± 0.88	17.88 ± 0.88	425.00 ± 37.75	0.81 ± 0.03

Values are mean ± standard deviation.

2.2. Determination of Soil Physicochemical Properties

The content of SOC was determined using an EA3000 C/N analyzer (Vector, Italy, Milan) after the soil was passed through a 100-mesh sieve and dried [24]; The dissolved organic carbon (DOC) was determined using fresh soil from which the roots and gravel had been removed. The soil was then passed through a 5 mm sieve. A 30 g sample of fresh soil was weighed and added to 50 mL of 1 mol·L^{−1} potassium sulfate (K₂SO₄). The mixture was placed on a shaker and agitated for 1 h to extract the DOC. The extract was analyzed using a JENA Multi 3100 C/N analyzer (Jena, Germany) [25]. The determination of mineral-associated organic carbon (MAOC) in soil adopted the classic physical wet fractionation coupled with chemical oxidation titration method. The specific procedures are as follows: Air-dried soil samples are gently crumbled and sieved through a 2 mm

mesh, with plant residual roots, stones and other impurities removed to prevent grinding from destroying the structure of soil aggregates. Sodium hexametaphosphate (NaPO_3)₆ at a concentration of 5 g/L is used as the inorganic dispersant. After adding a fixed amount of air-dried soil sample, the mixture is subjected to constant-temperature reciprocal shaking for 16 h to fully dissociate soil water-stable aggregates. The shaken mixed solution is wet-sieved through a 53 μm nylon sieve. The suspension passing through the sieve is the fraction enriched with MAOC. The recovery rate was greater than 95%. The under-sieve suspension is collected, left to stand, centrifuged, dried at low temperature, ground and re-sieved. Finally, the external heating potassium dichromate oxidation titration method is used to determine the organic carbon content of MAOC [26]. Fresh soil was fumigated with ethanol-free chloroform in a vacuum-sealed system for 24 h, followed by extraction using 0.5 mol/L K_2SO_4 solution. Microbial biomass carbon (MBC) was calculated as the difference in extractable organic carbon between fumigated and unfumigated soils divided by the conversion factor of 0.45 [27]. Two portions of fresh soil were weighed. One was used to extract the K_2SO_4 , and the other was fumigated with chloroform. Both samples were incubated at a constant temperature of 20 °C for 7 days. The fumigated soil was then extracted with K_2SO_4 after the chloroform was removed. The contents of C and N of both extracts were determined using the Multi 3100 C/N analyzer [28]; The amount of Ca was determined by titration with EDTA [29]. An aliquot of the digested sample solution was adjusted to $\text{pH} \geq 12$ with NaOH, treated with triethanolamine to mask Fe^{3+} and Al^{3+} , and titrated with standard EDTA solution (0.01 mol·L^{−1}) using Calcon (NN) indicator, with the endpoint recorded as the color change from wine-red to pure blue [22]; The available K was determined by ammonium acetate extraction-flame photometry [30].

2.3. High-Throughput Extraction and Sequencing of the Soil-Derived DNA

After the total soil DNA had been extracted, its quality was assessed by 2% agarose gel electrophoresis [31]. The 16S rRNA gene of the soil bacteria was amplified by PCR using primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') to target the V4-V5 variable regions. Primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the fungal ITS1–ITS2 regions [32]. The amplification protocol was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. The final extension was conducted at 72 °C for 10 min [33]. The PCR product was recovered using a 2% agarose gel, eluted with Tris-HCl at a concentration of 10 mmol/L and a pH of 8–8.5, and detected by 2% agarose gel electrophoresis [34]. The product was detected and quantified using a Quantus Fluorometer (Promega, Madison, WI, USA) [35]. The purified extended fragments were used to construct the PE300 library as described by the Illumina MiSeq platform (Illumina, San Diego, CA, USA) and then tested on this platform [36].

2.4. Statistical Analysis

The physical and chemical soil properties and the charts were drawn using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) [37]. The data were analyzed using SPSS 22.0 (IBM, Inc., Armonk, NY, USA) [38]. Alpha-diversity was assessed using three indices: Pielou evenness, Richness and Shannon. These indices were calculated in QIIME 2.15.3 and visualized via the R package ggplot2 2.15.3 [39]. One-way analysis of variance (ANOVA) was used to detect significant differences at $p < 0.05$ [40]. Where significant differences existed, Duncan's post hoc test was applied for multiple comparisons. Beta-diversity based on Bray–Curtis dissimilarity was calculated using QIIME 1.9.1 [41]. One-way ANOVA combined with Duncan's multiple range test (DMRT) ($p < 0.05$) was used to compare

relative abundances at the phylum level [42]. Partial least squares path modeling (PLS-PM), run with the R plspm package, was applied to clarify the associations among soil physicochemical properties, microbial communities, and soil processes [43]. Path coefficients and coefficients of determination (R^2) were estimated and validated via bootstrap resampling with 1000 iterations [43]. The overall model performance was further evaluated using the goodness-of-fit index [43].

3. Results

3.1. Physicochemical Properties of Soil in Poplar Plantations of Different Ages

The late-middle-aged stand had the highest contents of DOC, MBC, and SOC of 23.55 mg kg⁻¹, 151.57 mg kg⁻¹, and 9.48 g kg⁻¹, respectively, in the soil carbon pool (Figure 1a–c). However, the late-middle-aged stand had the lowest content of MAOC, while the middle-aged stand had the highest (Figure 1d). The contents of MBC increased significantly in the older trees, while the MAOC exhibited an inverted “V” shape. These results indicate that the labile components of carbon, including the DOC and MBC, are abundant in the late-middle-aged stand, while the stable carbon components, such as the MAOC, largely accumulated in the middle-aged stand. The contents of Ca and K in the soil differed among the different tree ages, and both decreased as the trees aged (Figure 1e,f). This suggested that the contents of mineral nutrients in the soil decrease with the development of the stand.

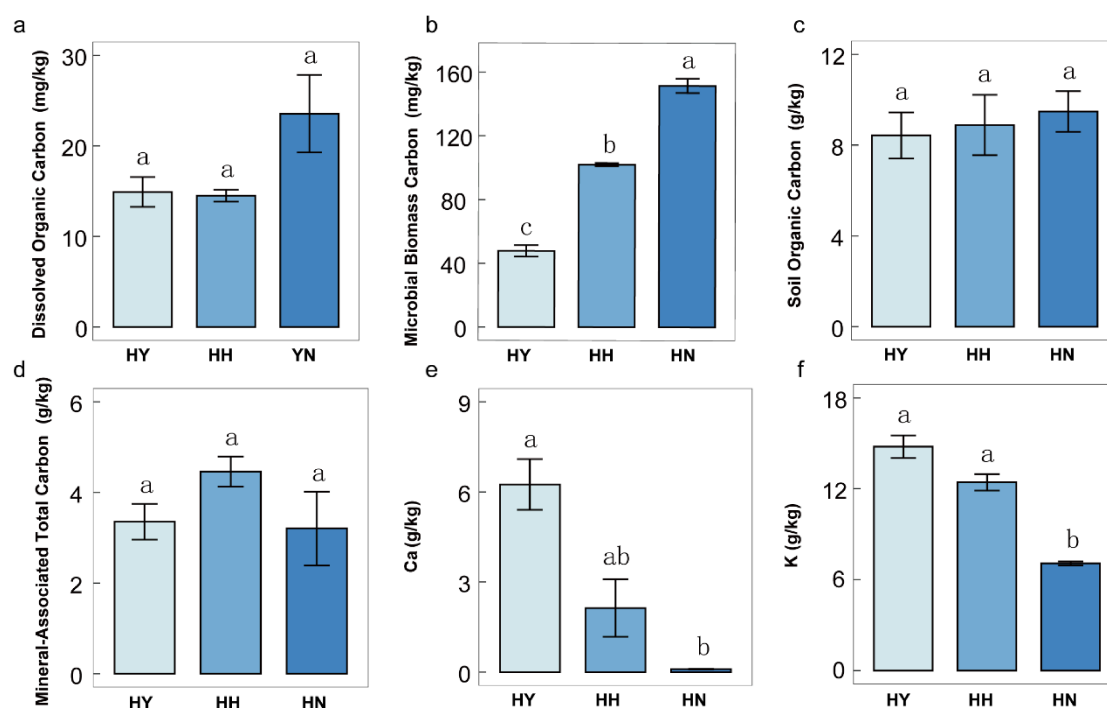


Figure 1. Physicochemical properties of soil in poplar plantations of different ages. (a) Soluble organic carbon; (b) Microbial biomass carbon; (c) Organic carbon; (d) Total mineral-associated carbon; (e) Calcium; (f) Potassium. Error bars, $n = 3$. HY: young plantation, HH: middle-aged plantation, HN: older plantation. Different lowercase letters indicate significant differences among stand age groups based on one-way ANOVA and Duncan’s post hoc test at $p < 0.05$.

3.2. Alpha and Beta Diversity of Soil Microbial Communities Across Poplar Plantations of Different Stand Ages

Significant variations were observed in Pielou evenness, Richness and Shannon index across poplar stands with different stand ages, with all metrics presenting a V-shaped trend (Figure 2a–c). In the fungal communities, the trends in evenness, richness, and Shannon

index of the different forest stands were similar to those of the bacterial communities (Figure 2d–f). Among them, the soil bacterial community in the late-middle-aged stand had the highest evenness, richness, and Shannon index, as well as the highest soil fungal richness. In contrast, the soil fungal community in the young stand had the highest evenness and Shannon index. This indicates that the bacterial diversity was the highest in the late-middle-aged stand, while the fungal diversity was the highest in the young stand. The bacterial and fungal diversities were both the lowest in the middle-aged stand. In the principal component analysis (PCA) of the bacterial communities, PC1 and PC2 accounted for 38.83% and 32.14% of the total variance, respectively, while PC1 and PC2 accounted for 23.51% and 18.14% of the total variance of the fungal communities, respectively. Moreover, the ANOSIM analysis revealed significant differences in the community composition among different forest stands for both the bacterial (ANOSIM: $R = 1$, $p < 0.003$) and fungal (ANOSIM: $R = 0.654$, $p < 0.019$) communities, with more pronounced differences in the bacterial communities than the fungal communities.

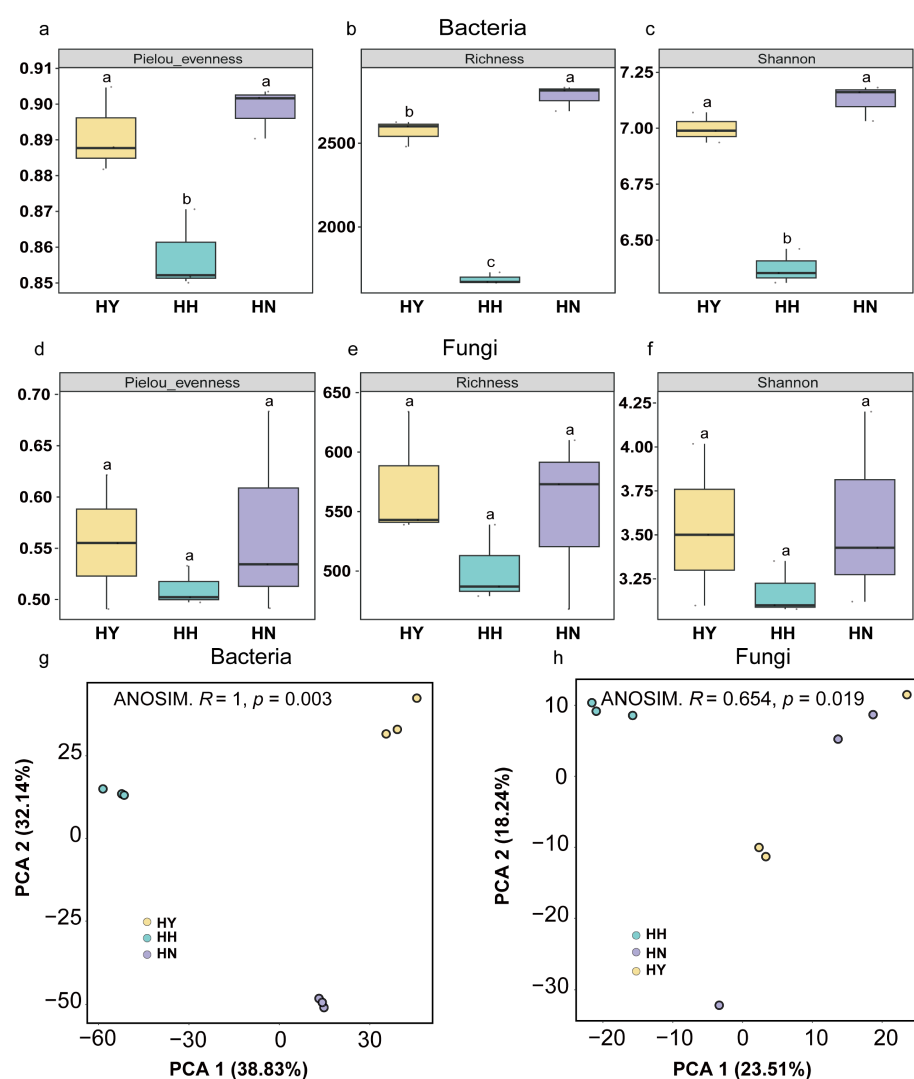


Figure 2. Analysis of the α -diversity and β -diversity of the soil microbial communities in different forest stands. (a,d) Pielou evenness; (b,e) Richness; (c,f) Shannon index; (a–c) bacterial communities and (d–f) fungal communities; (g) Principal Component Analysis (PCA) of the bacterial communities; (h) PCA of the fungal communities. Lowercase letters, degree of difference. $n = 3$. HY: young stand, HH: middle-aged stand, HN: late-middle-aged stand. Different lowercase letters indicate significant differences among age classes at $p < 0.05$; groups sharing the same letter are not significantly different.

3.3. Composition of the Soil Microbial Communities in Poplar Trees of Different Ages

An analysis of the soil microbial community composition of three different ages of poplar trees showed that in the soil bacterial community, the middle-aged stand was dominated by the phylum Acidobacteria, which accounted for 50.42%. The young and late-middle-aged stands were dominated by the phylum Proteobacteria, which accounted for 27.41% and 23.07%, respectively. For Acidobacteria, the relative abundance of Acidobacteria_Gp1 was higher in middle-aged poplar stands than in the young and late-middle-aged stands (Figure 3a). The soil fungal community was dominated by Ascomycota and Basidiomycota. Specifically, the relative abundances of Basidiomycota and Agaricomycetes decreased in late-middle-aged stands, whereas those of Ascomycota, Leotiomycetes, and several other fungal taxa increased. In addition, Agaricales and Inocybaceae showed the highest relative abundances in middle-aged stands (Figure 3c,d). Overall, the fungal community comprised fewer phyla than the bacterial community. Together with the observed age-related shifts in bacterial taxa, this pattern suggests that soil bacterial communities may exhibit broader taxonomic responses to habitat changes associated with poplar stand development.

3.4. Soil Microbial Network Analysis Driven by Tree Age

To further study how the stand age affects the co-occurrence patterns of soil microbiota, the co-occurrence networks of 200 key Amplicon Sequence Variants (ASVs) were assessed in the bacterial and fungal communities across the different stand ages (Figure 4a–f). The middle-aged stand had the largest number of edges (13,978) in the bacterial networks and also maintained a high node count of 200, with a positive-to-negative edge ratio of 11:10. In contrast, the late-middle-aged stand had the lowest number of edges (12,676). However, there was a higher network centralization degree in the late-middle-aged stands than in the other two forest stands (centralization degree = 0.099). The dominant bacterial phyla in the soil also changed as the forest stand developed. These results suggest that the interactions within the bacterial community are more complex in the middle-aged stand, and this change may be due to the change in the dominant bacterial phylum in the middle-aged stand to Acidobacteria. In the fungal networks, Ascomycota was the dominant fungal phylum in all three forest stands. The middle-aged stand continued to exhibit the highest number of edges (12,874), with a positive-to-negative edge ratio of 7:5. Its network centralization index was the greatest among all of the stand types, indicating that the fungal community in the middle-aged stand is characterized by the most intricate biotic interactions. But this pattern requires validation with larger sample sizes. Further study revealed that there were more edges in the bacterial networks than in the fungal networks for the same stand age.

Further analyses were conducted on the indicator species and network modules to determine whether certain soil microorganisms exhibited similar responses to different ages of poplar trees. The network of late-middle-aged stands had the highest number of nodes and the densest connections, indicating more complex interactions among the soil microbial groups and stronger associations within the community. Proteobacteria and Ascomycota, among other phyla, served as the core nodes of the network and drove the interactions within the community (Figure 5a). The microorganisms in the late-middle-aged stands were the most abundant in Module M1, and Acidobacteria and Basidiomycota were the dominant phyla. This reflects the strongest internal associations within Module M1 of the late-middle-aged stands, which may be related to the synergistic degradation of complex organic matter by saprophytic/symbiotic fungi. The abundance of microorganisms decreased and then increased in the older trees in Module M2. Module M2 exhibited a pattern similar to that of Module M1; however, its highest relative abundance was observed in young stands, with

Chloroflexi and Basidiomycota as the dominant phyla. In contrast, the relative abundance of Module M3 showed an inverted V-shaped pattern with increasing stand age, reaching its maximum in middle-aged stands, where Acidobacteria and Basidiomycota were the dominant phyla (Figure 5b).

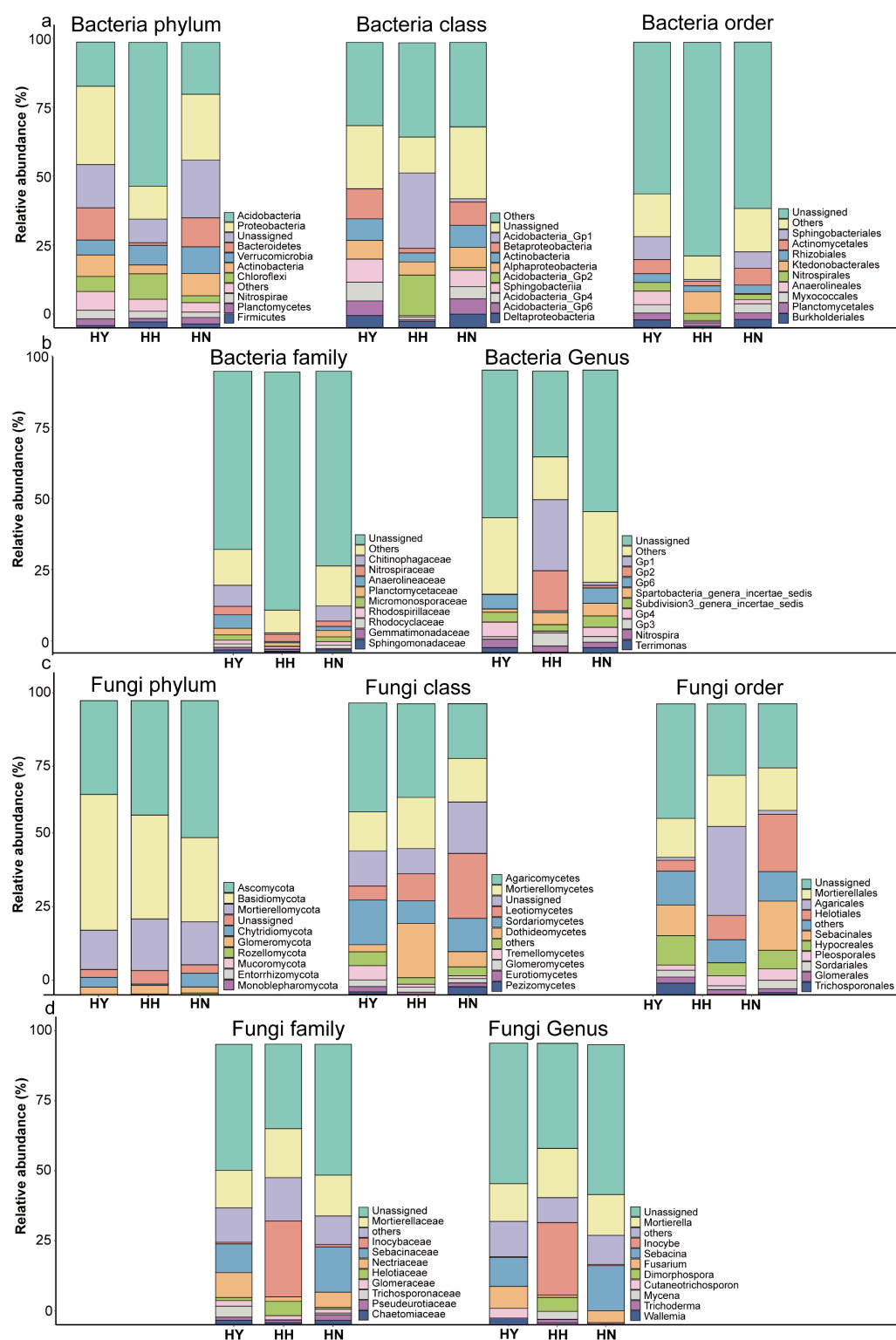


Figure 3. Composition of the microbial communities in different forest stands: (a) Bacterial phylum and class; (b) Bacterial order and family; (c) Fungal phylum and class; (d) Fungal order and family. HY: Young stand; HH: Middle-aged stand, HN: Late-middle-aged stand.

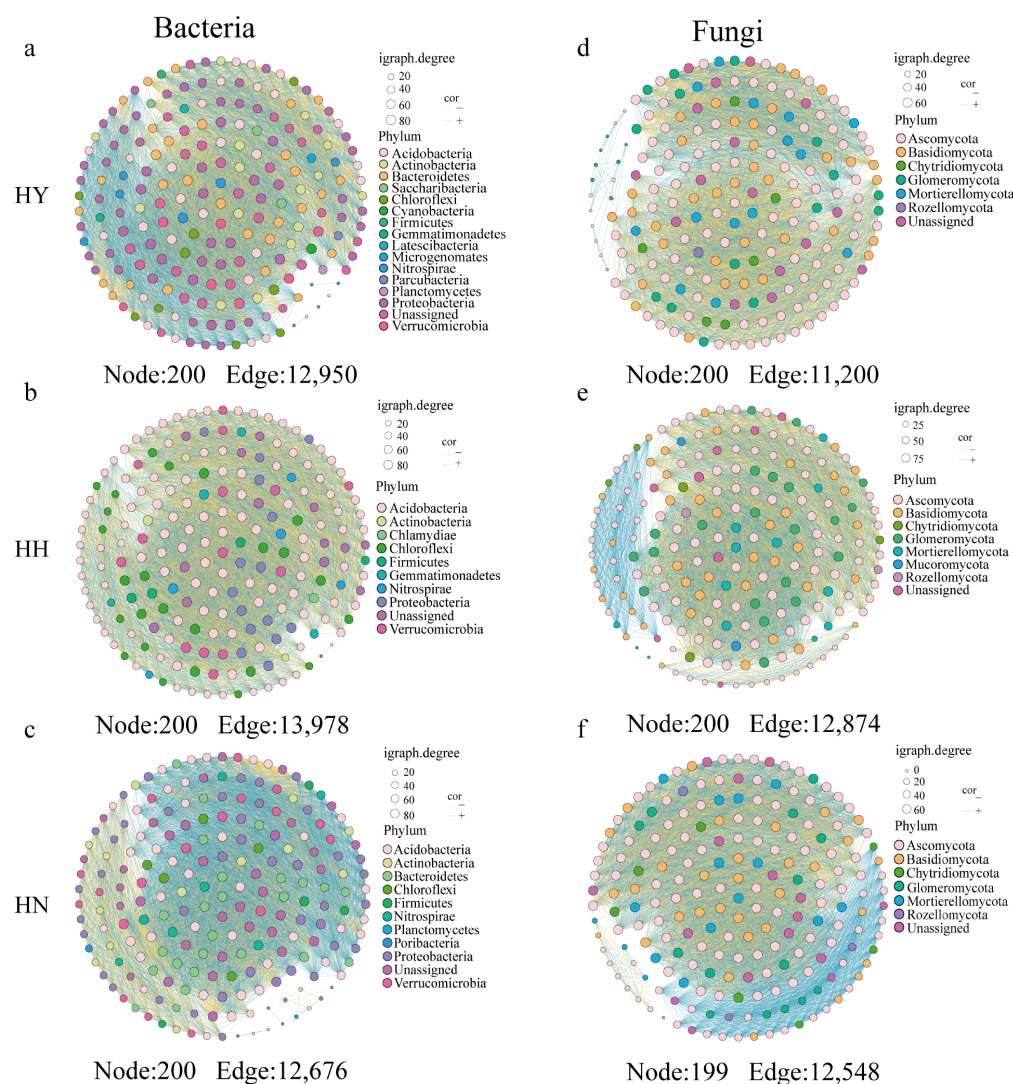


Figure 4. Co-occurrence network analysis. Bacterial communities: (a) HY, (b) HH, (c) HN; Fungal communities: (d) HY, (e) HH, (f) HN. The plots visualize taxonomic ranks via point color and size, the compositional differences in microbial communities. HY: Young stands; HH: Middle-aged stands; HN: Late-middle-aged stands.

3.5. The Ecological Assembly Process of the Soil Microbial Communities

Neutral Community Models (NCMs) were employed to analyze the assembly processes of soil microbial communities under different treatments (Figure 6a,e). In the bacterial communities, differences in the fitting curve R^2 and gene flow Nm values among the three forest stands reflected the distinct community structures during the stand development. The pie charts show that the proportion of “low” was the highest within each group, while “mid” was virtually absent. The beta nearest taxon index (β NTI), which measures the ecological processes of community assembly was ≥ 2 for all three forest stands. The highest value of 5.7 was identified in the late-middle-aged stand. This indicated that the phylogenetic turnover in the bacterial communities across the three stands was significantly higher than would be expected randomly. This suggested that the deterministic processes dominate the community assembly and that the bacterial community assembly in the late-middle-aged stand is more strongly influenced by deterministic processes (Figure 6b). Bray–Curtis-based Raup–Crick (RCbray) reflects the contribution of ecological processes to community turnover, and its values were ≤ -1 for all three forest stands. This indicated that the differences in species composition among the communities are primarily driven

by dispersal processes (Figure 6c). These results suggest that the turnover of pieces in the bacterial communities of the three forest stands is influenced by both environmental factors and dispersal processes. The importance of each ecological process was quantified in more detail by an iCAMP analysis (Figure 6d). Homogenizing dispersal was highest in the young stand (33.3%) and zero in the other two groups. However, there was high heterogeneous selection in each group, which indicated significant environmental heterogeneity in the different groups. In the fungal communities, the late-middle-aged stand had the highest Nm, indicating that this stand was more strongly influenced by random dispersal. Additionally, the proportion of highly abundant species in the young stand was 28.3%, which was higher than that in the middle-aged stand (18.7%) and the late-middle-aged stand (22.1%). This suggests that more “dominant species” drove the community assembly in the young stand (Figure 6e). In the young stand, $|\beta\text{NTI}| < 2$ and $\text{RCbray} > 1$ indicated that the fungal community assembly is primarily driven by stochastic processes, and dispersal limitation also played a role. The iCAMP analysis also found that both dispersal limitation and heterogeneous selection accounted for 33.3%. In the middle-aged and late-middle-aged stands, $\beta\text{NTI} > 2$ and $\text{RCbray} > 1$, indicating that fungal communities in these stands are primarily influenced by deterministic processes, and heterogeneous selection was dominant in both (Figure 6f–h).

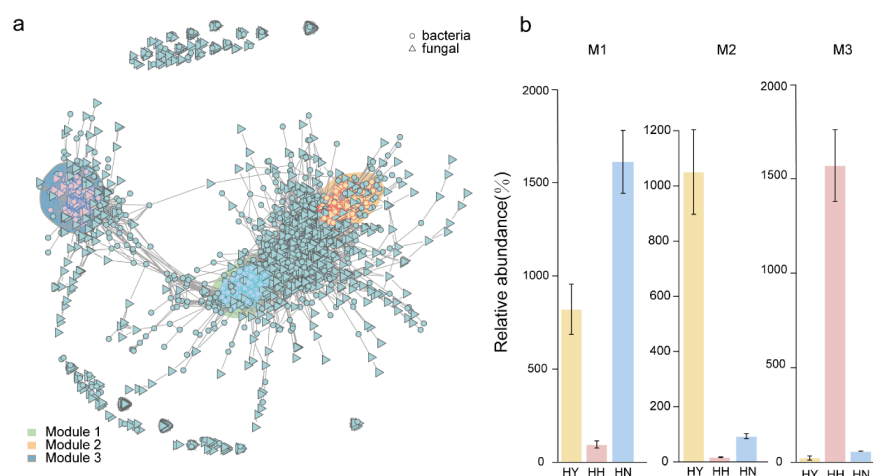


Figure 5. Co-occurrence network structure and module composition of soil microbial communities across stand age classes. **(a)** Interkingdom co-occurrence network of soil bacterial and fungal ASVs. Hollow circles represent bacterial taxa; hollow triangles represent fungal taxa. Nodes clustered with matching background colors belong to distinct network modules: light green = Module 1 (M1), orange = Module 2 (M2), light blue = Module 3 (M3). Gray lines indicate significant pairwise correlations between microbial taxa. **(b)** Relative abundance (%) of taxa assigned to Module 1 (M1), Module 2 (M2), and Module 3 (M3) across three poplar stands. Within each subplot, yellow bars = HY group, pink bars = HH group, light blue bars = HN group; error bars denote standard error of the mean. $n = 3$, HY: young stand, HH: middle-aged stand, HN: late-middle-aged stand.

3.6. Linkages Among Soil Physicochemical Properties, Soil Bacterial Communities, and Stand Age in Black Poplar Plantations

The relationships between the soil physicochemical properties, microbial communities, and different age groups were analyzed by a PLS-PM analysis (Figure 7a,b). The overall model fit was 0.61 in the bacterial community. Variations in age class exerted prominent inhibitory effects on soil Ca and K contents. Soil bacterial community composition and the strongest positive impact on β -diversity (Figure 7a). The impact of different age groups on the composition of the soil bacterial community had the strongest negative effect (−1.0401). In the fungal community, the overall model fit was 0.59. The β -diversity had a strong negative impact on the fungal community composition in the soil (−0.8135), and the

contents of Ca and K in the soil had the strongest positive impact on β -diversity (1.0543, Figure 7b). The path coefficients were calculated as -1.0401 and 1.0543 . Values outside the typical range of -1 to 1 suggest potential multicollinearity between variables and instability of the path model. Therefore, these results should be interpreted with caution. Moreover, compared with the bacterial communities, the different age groups of fungal communities had a stronger positive impact on the α -diversity (0.9393). The contents of Ca and K in the soil had a strong negative impact on the soil bacterial community composition in the bacterial communities, while they had a weak positive impact on soil fungal community composition in fungal communities. In addition, the β -diversity of both the bacterial and fungal communities was positively affected by the contents of Ca and K in the soil and by different age groups. The different age groups of poplar trees had the most significant negative impact on the contents of Ca and K in the soil ($p < 0.01$).

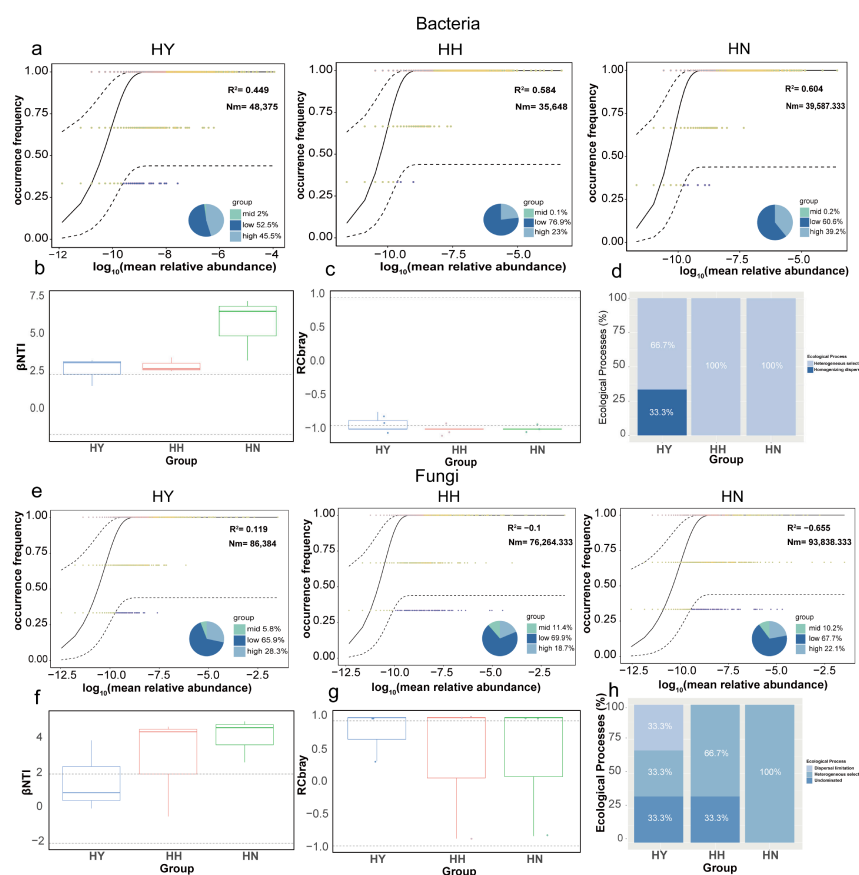


Figure 6. Community assembly processes of soil bacterial and fungal communities across different stand ages of black poplar plantations. (a) Fitting of the neutral community model to soil bacterial communities. The black solid line represents the best-fit neutral community model prediction; the upper and lower gray dashed lines denote the 95% confidence intervals around the neutral model fit. The colored horizontal solid lines separately mark the abundance thresholds dividing low-, medium-, and high-abundance taxa, corresponding to the pie chart categories in each subplot. (b,c) Boxplots of the β -nearest taxon index (β NTI) and Bray–Curtis-based Raup–Crick metric (RCbray) for bacterial communities. β NTI > 2 indicates dominant deterministic selection, while $|\beta$ NTI| < 2 combined with RCbray < -1 signifies homogenizing dispersal (stochastic process). (d) Relative contributions of dispersal limitation, heterogeneous selection, and homogenizing dispersal to bacterial community assembly across different stand age classes. (e) Fitting of the neutral community model to soil fungal communities. (f,g) Boxplots of β NTI and RCbray for fungal communities. (h) Relative contributions of dispersal limitation, heterogeneous selection, homogenizing dispersal, and neutral processes to fungal community assembly across different stand age classes. HY: Young stand, HH: Middle-aged stand, HN: Late-middle-aged stand.

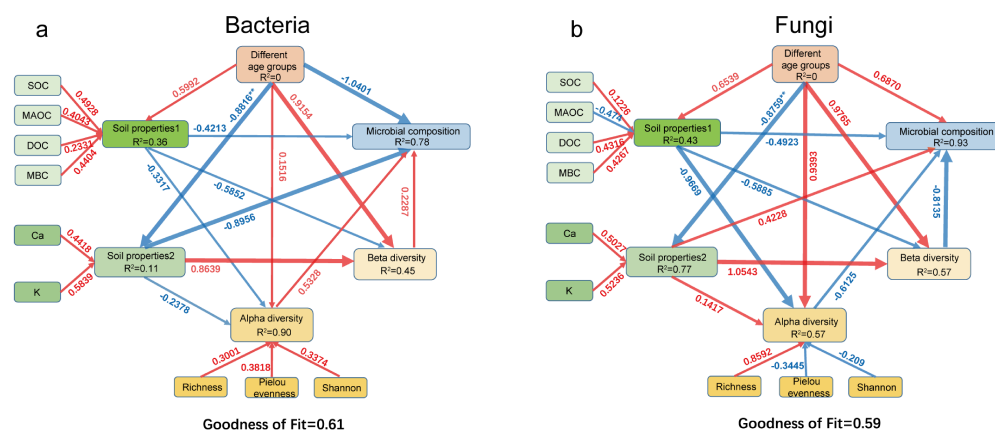


Figure 7. Relationships among soil physicochemical properties, soil microbial communities, and stand age in black poplar plantations based on partial least squares path modeling (PLS-PM). (a) Bacterial community model. (b) Fungal community model. Soil properties 1 included SOC, MAOC, DOC, and MBC; soil properties 2 included Ca and K; alpha diversity included richness, Pielou’s evenness, and the Shannon index. Ca, calcium; DOC, dissolved organic carbon; K, potassium; MAOC, mineral-associated organic carbon; MBC, microbial biomass carbon; SOC, soil organic carbon. Red and blue solid arrows represent statistically significant positive and negative causal relationships, respectively, with the values on the arrows indicating significant standardized path coefficients. ** indicates $p < 0.01$. The thickness of each arrow is proportional to the absolute value of the standardized path coefficient, with thicker arrows indicating stronger associations. Because the model was constructed using a limited sample size ($n = 9$), the results should be interpreted as exploratory associations rather than direct causal pathways.

4. Discussion

4.1. The Mechanism by Which the Tree Age Affects the Storage of Soil Carbon

The soil carbon pool represents the largest terrestrial carbon reservoir and plays a critical role in climate regulation and soil quality maintenance [44]. The results of this study indicate that stand age significantly influences the distribution of soil carbon fractions, including both labile and stable carbon pools, as well as mineral nutrient contents. Furthermore, the responses of these carbon fractions and nutrient elements varied among developmental stages, reflecting differences in carbon inputs, physiological characteristics of poplar trees, and interactions among plants, soil properties, and microbial communities. Regarding the components of the soil C pool, the component of active C generally increased in older trees and reached its peak in the late-middle-aged stands. This is primarily associated with the intensity and changes in quality of the C input at different stages of poplar growth [45]. Young stands are characterized by vigorous growth but produce a relatively small amount of litter that is easily decomposed [46]. Their root systems expand rapidly. Yet most of the C is inputted through fine roots with short turnover periods. As a result, there is a limited accumulation of active C [47]. The variation of stable C components exhibits an inverted “V” shape, which reaches its peak in the middle-aged stand. This is closely related to the unique ecological characteristics of the middle-aged stand. The depth of distribution and breadth of poplar root systems in the middle-aged stand reach their maximum values, and the soil environment most actively exchanges substances [48]. At this point, there is a moderate input of litter and a relatively low level of lignin. This makes it easier for microorganisms to decompose the soil C into small molecules of organic matter [49]. These organic compounds are more likely to combine with the particles of soil minerals to form a stable pool of C. The increase in the content of lignin in the litter of late-middle-aged stands [50], enhances the difficulty of decomposition. This, in turn, reduces the microbial transformation efficiency of C, which leads to a decrease in the

accumulation of MAOC. In summary, the tree age drives the dynamic differentiation of the components of the soil C by regulating the input and quality of litter from poplar, the intensity of root C input, and the efficiency of microbial transformation [51].

4.2. Ecological Differentiation of the Soil Microbial Community Composition Driven by Tree Age

Soil microorganisms constitute an indispensable functional component of forest ecosystems [52]. Changes in soil microbial community composition have been recognized as one of the most sensitive early indicators of soil quality variation across diverse ecosystem processes [53]. Existing studies have demonstrated that the α -diversity of soil bacterial communities exhibits a positive V-shaped trend along stand age gradients, reflecting phased fluctuations in microbial niche width and interspecific competition intensity. Specifically, young and late-middle-aged stands are characterized by relatively broad microbial niches and weak interspecific competition [54]. The niche contracts in middle-aged stands, and the competition intensifies. This demonstrates the ability of microorganisms to adapt to environmental changes during the development of stands. Young stands have rich soil nutrients but high spatial heterogeneity, and deterministic processes dominate. *r*-Strategist bacteria, such as Proteobacteria, proliferate rapidly, which results in a community structure characterized by high richness but low evenness. During the middle-aged stand stage, the environmental filtering effects are enhanced, and oligotrophic groups such as Acidobacteria gain an advantage; this leads to a temporary decrease in diversity. In the late-middle-aged stand stage, the soil DOC increases, and the pH decreases slightly. This expands the niche again and consequently increases diversity. Therefore, the differentiation of microbial community composition is achieved through “environmental filtering effects” by the alteration of the quality of soil C pools (labile C/stable C) and nutrient levels by tree age [10].

4.3. The Regulation of Tree Age on the Microbial Co-Occurrence Networks and Ecological Assembly Processes

Analyses of microbial co-occurrence networks and ecological assembly processes reveal that stand age profoundly shapes soil microbial interaction patterns and community assembly mechanisms [55]. For bacterial communities, middle-aged stands exhibit the maximum number of network edges (13,978), indicating the closest interspecific associations and the most complex bacterial interaction networks among all stand stages. This phenomenon is closely associated with the stable microenvironmental conditions in middle-aged stands, as evidenced by higher stable carbon content and sufficient nutrient supply in the soil [56]. The sophisticated network structure facilitates efficient microbial resource utilization via synergistic metabolic interactions [57]. For example, different bacterial groups can divide the labor to decompose different C components. This enhances the stability of the ecosystem. The fungal network also has the highest number of edges in the middle-aged stands. It contains unique Mucoromycota, which further confirms the “richness and stability” of the microbial community in the middle-aged stands. In contrast, the late-middle-aged stands have the fewest nodes, which may be related to the habitat filtering caused by the high content of lignin in the litter of the late-middle-aged stands [58].

During the ecological assembly process, the heterogeneous selection of bacteria dominates across all the tree ages, indicating that soil heterogeneity is the core driving force that shapes the bacterial community assembly [59]. The homogenization diffusion of young stands accounts for 33.3%, which may be related to the increased spatial homogeneity of the soil environment in young stands; this promotes the diffusion and colonization of microorganisms [60]. In fungal communities, the median β NTI values of the middle-age stands and older-young stands are both close to 4, indicating that strong deterministic processes (environmental filtering) dominate their assembly [61]. This is related to the dependence of fungi on complex organic matter. The age of trees leads to the direct selection

of specific fungal taxa by litterfall. Young stands may be subject to dispersal limitation, this is probably owing to the restriction of fungal dispersal because of the instability of their habitats. This suggests that the increased complexity of microbial networks and the enhanced deterministic assembly processes in the middle-aged stands play a key role in the transformation of soil C (accumulation of stable C) [62]. The simplification of the late-middle-aged stand network and the enhanced diffusion effect may be more conducive to the preservation of active C [62]. This provides a new concept to optimize the microbial functions and enhance the carbon sequestration potential of plantations by regulating the structure of tree ages [10].

4.4. The Relationship Between Soil Physicochemical Properties, Soil Microbial Community Composition, Soil Microbial Diversity and Different Age Groups

Cumulative evidence has confirmed that stand age of poplar plantations is closely coupled with soil physicochemical properties, microbial community composition, and microbial diversity [63]. In this study, different age groups of poplar trees were found to have the most significant impact on the contents of Ca and K in the soil ($p < 0.01$). This indicates that as the poplar trees grow older, the differences in their ages drive the allocation of elements in the soil; this may be related to the differences in physiological and ecological processes, such as the root absorption and litter return of poplar trees [64]. In the microbial communities, the bacteria and fungi exhibit differential responses to the age groups of poplar trees. Within the bacterial communities, different age groups have the strongest negative impact on the soil microbial composition. This enables them to form a cascading drive network of “tree age–soil properties–diversity–community composition” (Figure 8). This is consistent with the characteristics that bacteria are more sensitive to the changes in soil nutrients [65], and their community assembly is more strongly influenced by environmental selection [66]. In fungal communities, the impact of different age groups on the α -diversity is more pronounced. The direct effect of β -diversity on the fungal community composition (0.687) is lower than that on the bacterial communities. Moreover, β -diversity exerts a strong negative influence on the fungal community composition. Therefore, their community structure is more influenced by differentiation in the β -diversity driven by resource quality. Additionally, for both bacteria and fungi, the β -diversity is strongly affected by the content of Ca and K in the soil, as well as tree age. This indicates that the tree age shapes the spatial heterogeneity of microorganisms by altering the soil microenvironment, such as levels of nutrients and the quality of C storage, thereby regulating the community composition [9]. This differential pathway reflects the functional divergence of bacteria and fungi in ecological roles. Bacteria are more involved in the rapid cycling of soil nutrients [67], while the fungi are more closely associated with the transformation of complex organic matter [68]. Ultimately, both respond to the changes in habitat driven by the tree age and maintain the balance of material cycling in the plantation ecosystem.

4.5. Limitations and Implications

This study has several important limitations. First, each age class included only three composite soil samples, which limits the statistical power and makes the network, assembly, and PLS-PM analyses exploratory, rather than definitive. Second, only the 0–15 cm surface layer was sampled, and carbon variables were reported as concentrations, rather than whole-profile stocks; therefore, the conclusions about carbon storage and long-term sequestration are necessarily limited. Third, the chronosequence did not include mature or over-mature stands beyond 15 years, and the 11–15 year class should be regarded as an older stage, rather than a full mature stage. Fourth, the stand age was partly confounded with habitat description, prior land use, planting density, and canopy closure, so age effects

cannot be fully isolated from site and management effects. Finally, the study did not directly measure litter chemistry, root traits, enzyme activities, microbial functional genes, metagenomes, or microbial carbon-use efficiency. Future work should combine deeper soil profiles, longer chronosequences (>15 years), replicated sites, bulk-density-based carbon stock calculations, litter and root chemistry, metagenomic or functional gene analyses, and experimental microbial validation to determine whether the taxonomic patterns observed here translate into functional changes in carbon cycling.

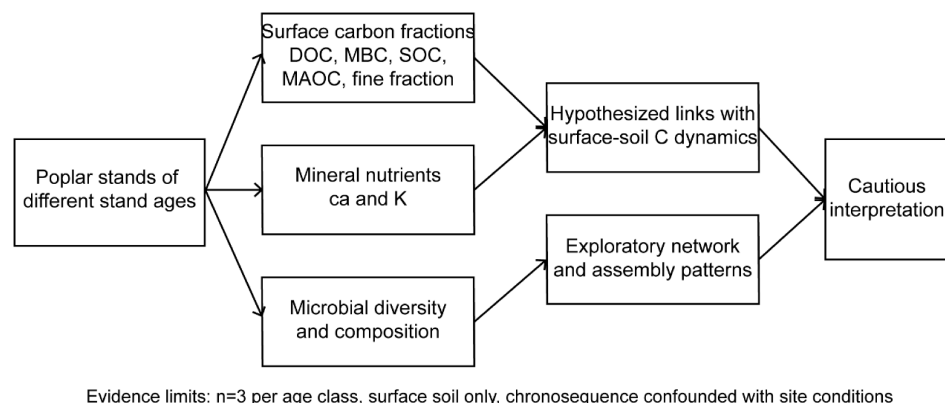


Figure 8. Conceptual framework linking early poplar stand development with surface-soil carbon fractions, measured mineral nutrients, microbial diversity and composition, and exploratory assembly/network patterns. The framework summarizes associations observed in this study and highlights the limits imposed by small sample size, surface-soil sampling, and chronosequence confounding.

5. Conclusions

This study shows that the early development of young poplar plantations (0–15 years) was associated with changes in surface-soil carbon fractions and microbial community structure. MBC increased toward the older stage, while DOC and SOC showed non-significant numerical maxima in the same age class. The operational fine-fraction/mineral-associated carbon variable showed a non-significant numerical maximum in the middle-aged stand, suggesting a possible transition stage for fine-fraction carbon formation. Bacterial and fungal communities differed among age classes, with Acidobacteria enriched in the middle-aged stand and Proteobacteria relatively more abundant in the young and late-middle-aged stands. Exploratory network and assembly analyses suggested stronger potential microbial associations in the middle-aged stand, but these findings require confirmation with larger datasets. Overall, the results indicate that early stand development is linked with coupled shifts in surface-soil carbon fractions and microbial community composition, while also emphasizing the need for deeper profiles, late-middle-aged stands, and functional validation before drawing conclusions about whole-plantation carbon sequestration.

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Data Availability Statement: All data generated or analyzed during this study are included in this published article. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under accession number(s) [PRJNA1441354] (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1441354> (accessed on 23 March 2026)).

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